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NEMATOLOGICA

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VOLUME I

EDITORIAL BOARD

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FOREWORD

Until the twentieth century the study of nematodes was almost a monopoly of physicians, veterinarians and parasitologists, working on parasites of man and animals, most of their work being taxonomical. Free-living nematodes in soil, fresh water and the sea were neglected because of their small size and hidden way of life. The few zoologists who did pay attention to them seldom found full employment in nematology. Plant parasitic nematodes also were more or less neglected. Some early workers drew attention to the genera *Meloidogyne* (the root knot nematode), *Anguina* (the wheat-gall nematode), *Heterodera* (cyst nematodes), and *Ditylenchus* (the stem and bulb nematodes), but progress in this field was hampered by the fact that nematodes are difficult to study and to handle and that diseases caused by them are obscure and slow to develop.

More recent work on nematodes, stimulated by progress in microscopical techniques, has stressed their rich differentiation in shape and form, their general occurrence in unexpectedly high populations in very different habitats:

> "Whether it be in utter darkness miles below the surface of the sea, or in some niche, brought for a little while above the freezing point once a year by the midsummer sun in the Antarctic Continent far beyond the range of any quadruped or bird, and, so far as we know, of any insect— not of course beyond the range of microscopic plants, for nemas are animals and must have organic food ..." (COBB)

It is being increasingly realised that nematodes play an important role in many obscure plant diseases. Crop rotation

in agriculture has resulted in larger yields all over the world, and in these increases of yield changes in nematode populations appear to play an important part.

Each year increasing numbers of scientists dedicate their time and efforts to identification, and to survey and experimental work on nematodes. The few professional journals on this field are overburdened with papers and publications are scattered throughout many periodicals, some of which are inaccessable. Hence the nematologists attending the Third International Symposium on "Plant Nematodes and the Diseases they cause" (Wageningen, 30 June—5 July 1955) approved the foundation of a new Journal, *Nematologica,* the first edition of which is presented herewith. The editors are indebted to Messrs. E. J. Brill, Leyden, for the proposal and offer to publish the journal.

Nematologica hopes to cover the field of nematology in general, except for papers on medical and veterinary subjects. The first two issues are reserved for the papers read at the Symposium mentioned above. In consequence, the contents of the first two issues will not be wholly representative of future issues. All papers read at the Symposium were relatively short and most of them were in the field of agricultural nematology. Taxonomy, physiology, morphology, genetics and other more fundamental research is lacking in these issues and figures of nematodes themselves are rare. It is our sincere aim that later issues will be different and will cover a wider field.

We feel encouraged by the kind cooperation of so many colleagues all over the world who are willing to support us as advisory editors and start *Nematologica* with the hope that it will prove to be a useful intermediary between nematologists of different countries, thus making a contribution to the advancement of science.

THE EDITORIAL BOARD

(*Nematologica*, Vol. I, 1956, no. 1)

OPENING ADDRESS OF THE NEMATOLOGY SYMPOSIUM 30 JUNE—5 JULY 1955

BY

C. J. BRIEJÈR

As Chief of the Division for Plant Protection of the Ministry of Agriculture, Fisheries and Food, it is an honour and a pleasure for me to welcome you in the name of that Ministry.

The directors of the inviting institutes, Prof Dr. A. J. P. OORT, Dr. J. G. TEN HOUTEN, and I myself are very glad that so many of you have been able to come to Wageningen. Originally we had been under the impression that only a limited number of specialists would attend these meetings. Thus, we had intended to meet in a much smaller room, but certainly there you would have felt like potato root nematodes in a cyst. Dr. P. A. VAN DER LAAN, Dr. M. OOSTENBRINK and Dr. J. W. SEINHORST, the organizers, have gone to much effort to change arrangements, so as to give you the feeling of free living eelworms in this Aula of the Agricultural University. However, we were all very glad that this necessity arose.

Most certainly we are all very much grieved that Dr. T. GOODEY is not with us. He was the man who organized the first Symposium at Harpenden in 1951. It is a pity he has not lived to see the fruitful results of his initiative. He has done a great deal for Nematology and I am sure we are all grateful for his efforts.

The meeting of the Nematology Section at the 1953 Zoological Congress in Copenhagen may be considered the Second Symposium. It was organized by our friend Dr. P. BOVIEN.

Now in 1955, two years later, we have the honour to have you here in Wageningen. The Symposium appears to have developed into a small congress. I think there have never been so many nematologists together before.

Allow me to take this opportunity to mention some of my personal views, you might call it my hobby horse. As you know, people having hobby horses are terrible people, for they take every opportunity to ride them.

The other day I read the absorbing book by David FAIRCHILD, entitled "The world was my garden". This book took me back to that fascinating second half of the nineteenth century, in

which so many wonders of Nature were discovered, so many treasures of Science dug up. The former generation has presented us with all those treasures, but never let us presume to know all or even much about them. We must handle them with utmost care and consideration. I am afraid this is not always done.

I myself am an entomologist, so let me take insects to stress my point. I am convinced we know very little about these creatures of which there are so many species in this world, and which also include some of our major pests today.

We make a mistake when we think we know much more than we really do. From this mistake the wrong is born, that is the wrong of combating insects with poisonous insecticides in a rather clumsy manner. And there you have my hobby horse. My view is that we ought to study the biology of insects much more than we do at present, in order to discover more refined methods of combating them.

Our present methods are very helpful for a rather short period, but in the long run they will result in disaster. I feel the same holds true for nematodes, except that here the danger may be still greater. Many nematodes live in the soil, soil about which we know very little. We do not know exactly what happens when we spray insecticides on plants. Still less do we know about the results of injecting pesticides into the soil. We must always realize that we are interfering with very complicated processes.

Therefore I am glad that we have succeeded to control potato sickness by crop rotation. This is a splendid start by a rather young science. I very much hope that you will work along this line as much as possible. Of course it may be that soil fumigants are necessary under the present circumstances, but let us consider this solution as temporary, not permanent.

Much work has to be done. You are here to exchange your views, to stimulate international cooperation and in general to develop your science.

I hope at the beginning of this Symposium that you will successfully find the right way and go in the right direction, listening attentively, and may I say humbly, to the great teacher called Nature.

Since I have many duties, I am sorry I can not be in your midst during the whole Symposium. It seems to be the fate of managing people to become immersed in many unfruitful worries, which often prevent them from taking part in really fruitful and interesting discussions.

(*Nematologica*, Vol. I, 1956, no. 1)

NEMATODENFORSCHUNG UND PFLANZENQUARANTÄNE

VON

H. GOFFART

(Inst. für Hackfruchtbau, Münster/Westfalen)

Es ist Ihnen als Fachkolleginnen und Fachkollegen geläufig, dass Nematoden mit Pflanzen und Pflanzenteilen aller Art verschleppt werden können. Dabei braucht es sich nicht nur um frische Pflanzenteile zu handeln. Auch Samen, Stroh, Heu und Kaff können ebensogut mit Nematoden behaftet sein, die sich hier jahrelang lebensfähig erhalten können. Eine besondere Gefahr stellen die cystenbildenden Nematoden dar, weil sie einmal eine noch grössere Widerstandsfähigkeit als die freilebenden Nematoden haben, zum anderen aber auch eine einzige Cyste oft mehrere hundert lebensfähige Larven und Eier enthält. Wie schnell cystenbildende Nematoden verbreitet werden können, zeigt das Beispiel des Kartoffelnematoden (*Heterodera rostochiensis*). Man kann zwar über den Ursprung des Kartoffelnematoden geteilter Meinung sein, aber es lässt sich nicht leugnen, dass seine Verbreitung über ganz Europa in erster Linie das Ergebnis einer Verschleppung, nicht nur mit Kartoffeln, sondern auch mit anderen bewurzelten Pflanzen und mit nicht einwandfreiem Verpackungsmaterial ist. Insbesondere sind es gebrauchte Säcke, in deren Gewebe Cysten sehr fest haften bleiben. So wurden Kartoffelnematoden in Jutesäcken bei der Einfuhr nach Amerika 1951 in 57 Fällen, 1952 ebenfalls in 57 Fällen, 1953 in 59 Fällen nachgewiesen. Vereinzelt wurden Cysten auch in Schiffsballast sowie im Reifenprofil eines alten LKW der Amerikanischen Besatzungsmacht gefunden, der aus Deutschland nach Amerika zurückgeschickt wurde. Aus den mir aus dem Jahre 1954 erst teilweise vorliegenden Ergebnissen ist bemerkenswert, dass von 21 Meerrettichsendungen aus Deutschland, 18 mit Kartoffelnematodencysten infiziert waren. Es ergibt sich hieraus die dringende Notwendigkeit, dafür zu sorgen, dass die für den Export bestimmten Sendungen soweit von Erde und anhaftenden Nematodencysten befreit werden, dass eine Verschleppung von Schädlingen nach menschlichem Ermessen ausgeschlossen ist.

Im Hinblick auf die deutschen Exportverhältnisse haben wir daher in den letzten Jahren Versuche durchgeführt, über die ich hier kurz berichten möchte.

Wie ich schon andeutete, fanden in den Jahren 1953 und 1954 mehrfach Verschiffungen von Meerrettich nach USA statt. Die Stangen wurden zwar, wie es die Vorschrift verlangt, durch Waschen von der anhaftenden Erde befreit, vielfach genügte aber diese Massnahme nicht, um auch alle Nematodencysten zu entfernen, wie sich bei der nachträglichen Untersuchung im New Yorker Hafen herausstellte. Wir versuchten daher, durch eine Warmwasserbehandlung von bestimmter Temperatur und Dauer zugleich auch die Cysten zu erfassen, ohne dass dabei die Meerrettichstangen geschädigt werden. Die Versuche

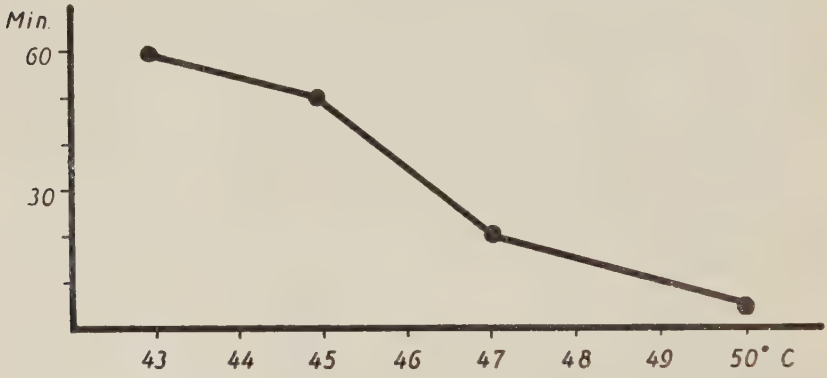


Fig. 1. Zum Abtöten von Kartoffelnematodencysten benötigte Zeit bei verschiedenen Wassertemperaturen.

wurden in der Weise angesetzt, dass wir die Cysten in kleine Beutelchen brachten und diese zugleich mit den Meerrettichstangen in das Warmwasserbad überführten. Die Lebensfähigkeit der Cysten wurde dann im Schlüpfversuch durch Zusatz von Wurzeldiffusaten ermittelt, während eine etwaige Schädigung der Meerrettichstangen durch Auspflanzen festgestellt werden sollte. Für das Abtöten der Cysten ermittelten wir bestimmte Temperaturwerte, die in Fig. 1 wiedergegeben sind und im wesentlichen durch den Linienverlauf dargestellt werden. Die Meerrettichstangen, die dieselbe Behandlung erfahren hatten, verhielten sich jedoch unterschiedlich. Mit Ausnahme der Behandlungsstufe 50° C 5 Minuten kam es in allen anderen Versuchsreihen zu einer Schädigung, die sich auf die Lagerfähigkeit der Meerrettichstangen ungünstig auswirkte. Nur die vorgenannte Warmwasserbehandlung tötete den Inhalt der Cysten ohne Schädigung der

Meerrettichstangen praktisch ab. Wenn nach der Behandlung wirklich einmal einzelne Larven nach Zusatz von Kartoffelwurzeldiffusaten noch ausschlüpften, so waren sie, wie wir nachweisen konnten, zur Cystenbildung nicht mehr imstande. Die nach der erwähnten Weise behandelten Meerrettichstangen blieben auch hinsichtlich ihrer Geschmacksschärfe unverändert.

In einem anderen Versuch wurde der Einfluss feuchter Wärme auf Meerrettichstangen und Kartoffelnematodencysten geprüft. Beide kamen in einen Thermostaten bei 50° C und 100% Luftfeuchtigkeit. Die Temperatur schädigte die Meerrettichstangen nicht, selbst wenn sie 60 Minuten einwirkte. Sie genügte aber nicht zum Abtöten der Nematodencysten. Bei Verwendung trockener Hitze war zum Abtöten der Cysten sogar eine 20 Minuten anhaltende Temperatur von 70° C erforderlich. Bei dieser Temperatur traten aber an Meerrettichstangen schwerste Schäden auf. Weder mit feuchter noch mit trockener Wärme ist also im vorliegenden Falle ein ausreichender Erfolg zu erzielen. Es ist also nur eine Warmwasserbehandlung wie oben geschildert geeignet, etwa anhaftende Kartoffelnematodencysten ohne Schaden für den Meerrettich selbst abzutöten. Um eine noch grössere Sicherheit zu erlangen, empfiehlt es sich, Meerrettichstangen, die kopfrissig sind, und daher mit grösserer Wahrscheinlichkeit Nematodencysten verschleppen können, überhaupt vom Export auszuschliessen. Selbstverständlich sollte nur Meerrettich zum Export vorgestellt werden, der auf nachweislich nicht nematodenverseuchtem Boden angebaut wurde.

Ein anderes Problem betrifft die Maiblumen, die in Deutschland besonders im Raume Hamburg, Lübeck und Süd-Lauenburg angebaut werden. Der Lübecker Maiblumenanbau ist als Familienkultur in Kleinstbetrieben bereits im Jahre 1830 entstanden. Die hier geernteten Blühkeime stellen einen beliebten Ausfuhrartikel dar. 1954 wurden aus diesem Gebiet rund 7300000 Treibkeime in die Exportländer Amerika, England, Frankreich, Holland, Schweiz und Skandinavien versandt. Der Maiblumenbau erfolgt auf Kleinflächen meist im Wechsel mit Kartoffeln, und dieses Gelände ist öfters mit Kartoffelnematodencysten infiziert. Als daher 1949 in Maiblumensendungen, die für USA bestimmt waren, erstmalig Cysten von *Heterodera rostochiensis* festgestellt wurden, erhob sich die Frage, auf welche Weise man die für den Export bestimmten Sendungen frei von Nematodencysten machen kann. Die ersten Versuche führten Richardson und Spruyt durch. Sie stellten fest, dass Eintauchen der Maiblumenkeime in warmes

Wasser von 48° C für 30 Minuten die beste Behandlungsweise ist, die von den Keimen gut vertragen wurde und gleichzeitig den Brutinhalt der Cysten abtötet. Derart behandelte Keime müssen aber bald ausgepflanzt werden. Erneutes Einfrieren und Einlagern nach einer Warmwasserbehandlung rief nämlich erhebliche Schäden hervor. Pape fand, dass selbst bei der genannten Temperatur die Blühfreudigkeit der Maiblumenkeime zurückging, wenn sie unmittelbar nach der Behandlung zum Treiben angesetzt wurden. Damit kommt der Warmwasserbehandlung nur eine begrenzte Wirkung zu, denn die für den Export bestimmten Sendungen gehen in eingefrorenem Zustand nach Übersee und werden oft erst Monate später abgetrieben. Bijloo ging daher von der Warmwasserbehandlung ab und prüfte die Frage, ob und in welchem Umfange das organische Beizmittel Aaventa einen Einfluss auf Kartoffelnematodencysten ausübt, ohne dabei die Maiblumenkeime zu schädigen.

Bei den Versuchsreihen, die mit 0,5 bis 4% Aaventa bei unterschiedlicher Behandlungsdauer durchgeführt wurden, lag der Schwellenwert für die nematizide Wirkung bei zweistündiger Einwirkung einer 4%-igen Lösung von Aaventa. Dieser Wert ging aber über das von den Maiblumen ertragene Mass bedeutend hinaus, die schon durch eine 1%-ige Lösung geschädigt wurden.

Wir haben ähnliche Versuche mit Aaventa und Aabulba im Spätherbst 1953 und 1954 durchgeführt. Wir prüften Kartoffelnematodencysten und Maiblumenkeime in drei verschiedenen Konzentrationen von unterschiedlicher Dauer, nämlich in 0,25% 3 Stden, in 0,5% 1,5 Stden und in 1% 0,5 Stden. Die Temperatur der Beiz-Flüssigkeiten betrug in allen Fällen 12° C. Nach der Behandlung wurden die Keime in fliessendem Wasser gründlich abgespült. Zum Vergleich wurden Kartoffelnematodencysten in kleinen Leinenbeutelchen und Maiblumenkeime 3 Stunden in Leitungswasser von 12°C eingetaucht. Aaventa und Aalbulba wirkten in einer 1%-igen wässrigen Lösung auf den Schlüpfvorgang der Nematodenlarven stark hemmend ein, die nematizide Wirkung des Aabulba war auch höher als die des Aaventa (Tab. I). Auf Maiblumenkeime übte aber schon 0,25% Aaventa eine erhebliche Schädigung aus, wenn die Keime nach der Behandlung in Moos verpackt bis zum Austreiben bei 6°C gelagert wurden (Taf. I und Tab. II). Werden sie aber zum Einfrieren gebracht, dann kommt es nur noch selten zum Austreiben. Auch wenn Maiblumen-Eiskeime erst nach dem Auftauen im Tauchbeizverfahren mit Aaventa behandelt wurden, zeigten sich erhebliche Schäden am Blattspross und in der Entwicklung der Blüten.

Aaventa

Aabulba

1%
0,5 Std.

0,5%
1,5 Std.

0,25%
3 Std.

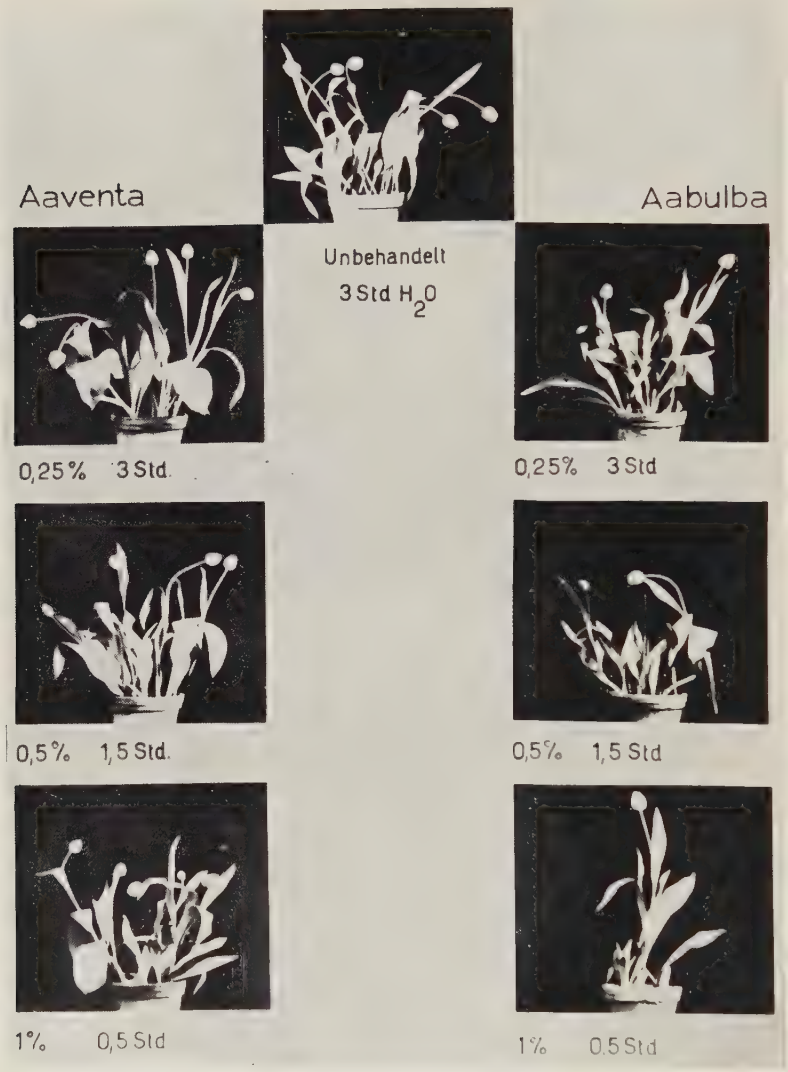
Unbehandelt
3 Std. H_2O

0,25%
3 Std.

0,5%
1,5 Std.

1%
0,5 Std.

Beizversuche an *Convallaria majalis*.



Beizversuche an Darwintulpen.

TABELLE I

Zahl der geschlüpften Kartoffelnematodenlarven in 40 Tagen

Aabulba	1 %	0,5 Std.	63
	0,5 %	1,5 „	231
	0,25%	3 „	296
Aaventa	1 %	0,5 Std.	149
	0,5 %	1,5 „	339
	0,25%	3 „	779
Unbehandelt		3 Std. H_2O	3557

TABELLE II

Beizversuche an Convallaria majalis

Behandlung			Durchschnittslänge des Blattsprosses in cm nach 22 Tagen	Blütenrispen normale	vertrocknete	keine
Aabulba	0,25%	3 Std.	3,5	0%	0%	100%
	0,5 %	1,5 „	3,9	0%	0%	100%
	1 %	0,5 „	3,4	0%	0%	100%
Aaventa	0,25%	3 Std.	10,7	0%	100%	0%
	0,5 %	1,5 „	9,0	0%	80%	20%
	1 %	0,5 „	7,0	0%	87%	13%
Unbehandelt		3 Std. H_2O	20,5	87%	10%	3%

Ein vorher mit dem Beizmittel Ceresan durchgeführter Versuch ergab bei den Blüten eine noch wesentlich höhere phytotoxische Schädigung. Die quecksilberhaltigen Beizmittel werden von den Maiblumen in erheblichem Umfang aufgenommen und anscheinend auch beträchtlich gespeichert, wodurch sich die starke phytotoxische Wirkung erklärt. Überhaupt scheinen Maiblumenkeime gegen chemische und physikalische Einflüsse ausserordentlich empfindlich zu sein. Sie ertragen selbst eine Warmwasserbehandlung von 48°C nur dann, wenn sie unmittelbar nachher ausgepflanzt werden. Hinsichtlich der für den Export bestimmten Maiblumenkeime muss es daher vorerst noch beim mechanischen Abspülen in Wasser bei Zimmertemperatur bleiben. Da dieses Verfahren aber auf die Cysten keinen Einfluss hat, wird man mehr denn je Wert darauf legen müssen, Maiblumen nur dann zum Export zuzulassen, wenn sie auf nachweislich nicht verseuchtem Boden gewachsen sind.

Wir haben die phytotoxischen Eigenschaften quecksilberhaltiger

Beizmittel bei verschiedenen Pflanzen untersucht. Sie sehen in der Fig. 3 die Wirkung von Aaventa und Aabulba auf Darwintulpen dargestellt, die mit 0,25%, 0,5% und 1% gebeizt worden sind. Die Kontrollpflanzen kamen wiederum für 3 Stunden in Leitungswasser von 12°C. Zunächst fällt auf, dass Aabulba auch hier in allen Fällen stärker phytotoxisch gewirkt hat als Aaventa. Vor allem zeigten die mit Aabulba gebeizten Zwiebeln oft ein gestauchtes und gedrehtes Wachstum, die Knospen blieben vielfach stecken, die Blüten waren anomal und die Blattnerven traten hervor. Wir erkennen aber auch schon deutliche Wachstumsdepressionen nach Behandlung mit 1% Aaventa, doch waren die Schäden nicht so erheblich wie bei Maiblumen. Was Sie sehen auf Taf. II, gibt Ihnen Tab. III in Zahlen

TABELLE III

Beizversuche an Darwintulpen

Behandlung			Länge d. Blattsprosses in cm nach 6 Tagen. Mittelwert	Zwiebeln: mit normaler Blüte	mit anomaler Blüte	ohne Blüte
Aabulba	0,25%	3 Std.	13-23 (16,9)	72%	28%	0%
	0,5 %	1,5 „	9-19 (15,0)	60%	40%	0%
	1 %	0,5 „	3-20 (11,8)	28%	64%	8%
Aaventa	0,25%	3 Std.	13-24 (18,0)	100%	0%	0%
	0,5 %	1,5 „	12-21 (17,7)	88%	12%	0%
	1 %	0,5 „	6-21 (17,0)	96%	4%	0%
Unbehandelt		3 Std. H_2O	15-23 (19,0)	100%	0%	0%

wieder. Vor allem sei auf die sehr unterschiedliche Länge der Blattsprosse und auf den geringen Anteil von Zwiebeln mit normaler Blüte nach dem Beizen mit Aabulba hingewiesen.

Derselbe Versuch wurde auch mit Traubenhyazinthen durchgeführt (Tab. IV). Er zeigt ebenfalls den stärkeren phytotoxischen Einfluss des Beizmittels Aabulba, z.B. in der Länge der Blattsprosse 6 Tage nach dem Ansetzen oder in der Länge der Blütenstengel bei Blühbeginn. Auch der Blühbeginn wird nach Beizen mit Aabulba etwas verzögert. Die Blätter waren namentlich bei 1% Aabulba zum grössten Teil gestaucht, gedreht und fleischig, bei Aaventa waren sie normal.

Sehr unterschiedliche, z.T. schwere Keimverzögerungen traten bei Kartoffelknollen auf. So ergaben Versuche, dass Pflanzkartoffeln der Sorten Bona und Lerche zu dem Zeitpunkt, als die unbehandelten

Versuchsreihen bereits zu 100% aufgelaufen waren, die mit Aaventa (0,25%) gebeizten erst zu 70 bzw. 84% erschienen waren. Bei Verwendung von Ceresan betrugen die Auflaufwerte 98 bzw. 94%. In einem anderen Versuch, bei dem die Kartoffelsorten Sieglinde und

TABELLE IV

Beizversuche an Muscari

Behandlung			Durchschnittslänge des Blattsprosses in cm nach 6 Tagen	Durchschnittl. Blütenstengelanzahl der Zwiebeln	Durchschnittslänge der Blütenstengel in cm bei Blühbeginn
Aabulba	0,25%	3 Std.	11,5	1,7	14,0
	0,5 %	1,5 „	9,2	1,8	9,3
	1 %	0,5 „	6,3	1,7	9,4
Aaventa	0,25%	3 Std.	13,7	1,9	13,5
	0,5 %	1,5 „	13,3	2,0	14,0
	1 %	0,5 „	12,9	1,9	14,1
Unbehandelt		3 Std. H_2O	12,9	2,3	14,0

Ackersegen verwendet wurden, lagen die Auflaufzahlen nach Beizen mit Albertan (0,25%) bei 8 bzw. 34%, nach Beizen mit Germisan (0,25%) bei 74 und 98%. Auch ein erst im letzten Jahr benutztes neueres Präparat, mit dem wir Beizversuche in 0,1% durchgeführt hatten, ergab in diesem Jahr schwerste Auflaufschäden. Gegenüber Blumenzwiebeln schien es allerdings weniger schädigend zu sein. Wir konnten es hier bisher nur in Konzentrationen von 1, 2 und 3% prüfen und stellten fest, dass die mit diesen Lösungen behandelten Zwiebeln von *Scilla campanulata* in der Zahl der Blütenrispen keine erheblichen Unterschiede aufzuweisen hatten. Es ist natürlich möglich, dass die gewählte Versuchspflanze gegenüber dem Mittel eine geringere Empfindlichkeit besitzt. Daher müssen Versuche noch mit anderen Pflanzen durchgeführt werden.

Zusammenfassend ergaben unsere Versuche zur Entseuchung lebender Pflanzen von anhaftenden Nematoden Folgendes:

1) Bei Meerrettich kann eine 5 Minuten anhaltende Warmwasserbehandlung von 50°C etwa anhaftende Nematodencysten abtöten, ohne dass die Meerrettichstangen geschädigt werden.

2) Die Anwendung nematizid wirkender Mengen quecksilberhaltiger Beizmittel führte bei Maiblumen zu schweren Wachstumsdepressionen

und zu einer starken Herabsetzung der Blühwilligkeit, namentlich. wenn die Pflanzen nach der Behandlung bis zum Auspflanzen noch eine Zeitlang gelagert werden. Aabulba besitzt eine höhere nematizide Wirkung als Aaventa, wirkt anderseits aber auch stärker phytotoxisch.

3) Kartoffelknollen sind gegenüber quecksilberhaltigen Beizmitteln sehr empfindlich und reagieren hierauf mit mehr oder weniger starken Keimverzögerungen.

4) Die Empfindlichkeit der Pflanzen gegenüber einem Beizmittel ist unterschiedlich. Sie ist nicht nur artspezifisch, sondern hängt auch von dem Zustand der Pflanzen z.Zt. der Behandlung und von den Massnahmen nach der Behandlung ab.

5) Bei dem augenblicklichen Stand unserer Kenntnisse wird man bei Pflanzen, die für den Export in Aussicht genommen sind, auf die Forderung ihres Anbaues auf nicht mit Nematodencysten verseuchtem Boden nicht verzichten können.

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DISCUSSION

E. Mühle: Es wird angeregt, die zu behandelnden Nematoden einen Vorbehandlung auszusetzen, um sie dadurch gegen die Beizmittel empfindlicher zu machen und die Beizkonzentrationen dadurch soweit herabsetzen zu können, dass die zu behandelnden Pflanzen keine Schäden mehr erleiden.

H. Goffart: Es soll der Anregung nachgegangen werden, doch wird darauf hingewiesen, dass jede Behandlung eine mehr oder weniger starke Stimulation des Pflanzenwachstums hervorruft, die sich pflanzenschädigend auswirken kann, wenn die Pflanzen nach der Behandlung noch einige Zeit gelagert werden, bevor sie ausgepflanzt werden.

J. H. Schuurmans Stekhoven: fragt ob Untersuchungen mit Getreide und Zwiebeln durchgeführt worden sind.

H. Goffart: Es wurden keine Versuche mit Getreide und Speisezwiebeln durchgeführt.

J. D. Bijloo: Bei unseren Versuchen zur Entseuchung von Saatkartoffeln gegen *Heterodera rostochiensis* haben wir mit den Quecksilbermitteln Aaventa, Aardisan und Aretan gearbeitet. Aaventa zeigte sich zu phytotoxisch. Aardisan ist weniger phytotoxisch für Kartoffeln als Aabulba. Aretan zeigte sich weniger nematizid als Aardisan.

Nach dem machinellen Waschen von Kartoffeln werden sie eine Minute lang

in eine 0,5% Lösung Aardisan getaucht und sofort in Säcke überbracht. Die in diese Säcke eingebrachten Cysten erwiesen sich gleich nach der Tauchung noch nicht abgetötet. Wenn man aber darauf achtet, dass die Säcke mit Kartoffeln wenigstens 7 Stunden feucht bleiben, werden die Cysten wohl abgetötet (Litteratur: Proeven ter Bestrijding van Heterodera rostochiensis door het wassen en ontsmetten van Aardappelen; Bijloo J. D., T. Pl. ziekten 61 (1955) 47-51.)

Die Kartoffeln werden in Säcken getrocknet und auf einen perforierten Boden gesetzt, wodurch Luft geblasen wird, wie man es auch beim Aufbewahren von Kartoffeln macht.

Es erwiesen sich Unterschiede in der Empfindlichkeit der verschiedenen Kartoffelsorten für die organischen Quecksilbermittel. Die frühe Sorte Eersteling zeigte sich stark geschädigt, die Sorte Bintje zeigte jedoch kaum eine Schädigung.

Spätere, noch nicht publizierte Versuche, zeigten, dass die Cysten auch abgetötet waren, indem wir folgendes probiert haben: Die Kartoffeln wurden eine Minute lang in 0,5% Lösung Aardisan getaucht, und eine Stunde getrocknet. wonach sie 3 Wochen trocken in Säcken aufbewahrt worden sind.

Das verdampfende organische Quecksilber scheint noch eine Nachwirkung zu haben und die Cysten abzutoten.

(*Nematologica*, Vol. I, 1956, no. 1)

APERÇU DES TECHNIQUES EMPLOYÉES DANS LES RECHERCHES SUR LE NÉMATODE DORÉ DE LA POMME DE TERRE

PAR

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Les problèmes en rapport avec les recherches et l'étude des moyens de lutte contre le nématode doré de la pomme de terre, ont nécessité le développement et la mise au point de différentes techniques. Vu que les techniques sont généralement plus ou moins différentes dans les laboratoires qui s'occupent d'un même problème, nous avons estimé utile de donner dans cet exposé un bref aperçu de nos méthodes de recherche en rapport avec les problèmes suivants:

1) l'analyse des échantillons de terre;
2) l'examen des fumigants quant à leur valeur nématicide;
3) l'examen des espèces de Solanum en ce qui concerne leur résistance;
4) la récolte de kystes et la production de la sécrétion radiculaire.

A. L'ANALYSE DES ÉCHANTILLONS DE TERRE

Ainsi qu'il est d'usage dans plusieurs autres laboratoires, nous obtenons les kystes des échantillons de terre au moyen d'une batterie de cruches de FENWICK modifiées, installées sur une table (Pl. III, fig. 1). A côté des améliorations déjà apportées par d'autres chercheurs, nous avons modifié le système en ce sens, que le matériel flottant n'est plus décanté par un jet d'eau le long de l'entonnoir se trouvant au dessus de chaque cruche, mais par un jet d'eau dirigé en sens vertical dans la cruche à travers une petite ouverture dans la paroi. A cette fin un petit tuyau courbé, d'un diamètre d'un centimètre, y est adapté à environ 15 cm en dessous du collet. Ce tuyau est raccordé à la conduite d'eau et il est introduit dans les cruches en évitant des parties saillantes à l'intérieur. Cette modification permet de rassembler en une seule fois et rapidement tout le résidu flottant.

A côté de cette modification deux autres détails sont encore à noter: notamment le montage de l'entonnoir et l'achèvement du fond des

cruches. L'échantillon arrive dans la cruche par un entonnoir en verre, placé à environ 3 cm au-dessus de l'ouverture de la cruche. Ceci a l'avantage que le matériel flottant ne peut plus adhérer au cou de l'entonnoir. Le fond des cruches est oblique vers l'ouverture de nettoyage, comme dans l'appareil d'OOSTENBRINK, et il est bombé, de façon que l'intérieur se nettoie de soi-même quand on enlève le bouchon. Un simple rinçage avec de l'eau propre suffit pour un nettoyage à fond. Ces différentes adaptations ont permis d'opérer d'une manière plus vite et plus exacte: un homme est capable de faire 24 échantillons de 200 gr par heure. Une série d'analyses effectuées avec des échantillons de différentes sortes de terre, artificiellement infectés avec un nombre connu de kystes, variant de 1 à 100, a démontré que tous les kystes peuvent être récupérés.

En résumé, l'examen qualitatif est effectué de la façon suivante: les échantillons arrivant au laboratoire dans des sachets en plastic d'une capacité de 500 gr de terre, sont séchés à l'air. Après séchage 200 gr de terre est introduite dans la cruche à travers un tamis à larges mailles au moyen d'un jet d'eau très fort. Le matériel montant étant rassemblé à la surface, il suffit d'ouvrir pendant un instant le robinet d'eau secondaire pour décanter le résidu. Ce résidu est receuilli et séché dans un petit tamis, répandu sur un petit bac de comptage selon FENWICK et examiné au binoculaire sur la présence de kystes.

Pour l'analyse quantitative les 100 premiers kystes sont mis sur un porte-objet en deux groupes de 50, et disséqués à l'aide d'un verre de pression d'OOSTENBRINK. Le contenu est transvasé dans un cylindre gradué et dilué jusqu' à 100 cm^3. Trois fois un cm^3 de suspension est compté à l'aide d'un petit verre de comptage du modèle B. G. PETERS.

B. EXAMEN DES FUMIGANTS QUANT À LEUR CARACTÈRE NÉMATICIDE

Les nématicides sont examinés en trois phases consécutives:

1) des essais „in vitro"
2) des essais en bacs;
3) des essais en pleine terre.

Nous nous limiterons dans cet exposé à la description des essais effectués en bacs, les essais „in vitro" ayant fait l'objet d'une publication antérieure. Ces bacs ont comme dimensions 25 cm x 25 cm x 30 cm, soit la superficie et la profondeur couverte par une injection en pleine terre (Pl. III, fig. 2). Ils sons remplis du type de sol à examiner et restent découvert pendant l'essais. Le produit y est introduit au milieu

à 20 cm de profondeur. Le degré et les zones de l'effet nématicide dans les bacs sont controlés au moyen d'essais d'éclosion effectués sur des kystes, qui ont été introduits à des points bien déterminés autour du point d'injection. Soixante kystes de diamètre et de contenu connus, sont mélangés avec de la terre et renfermés dans de petites cages de treillis métallique approprié. Ces cages sont installées à huit places différentes dans le bac, variant des plus favorables au moins favorables vis à vis du point d'injection. Après le traitement les cages sont récupérées et numérotées, les kystes en sont isolés, lavés, divisés en trois parties egales et soumis à l'action de la sécrétion radiculaire dans des verres à éclosion. L'éclosion des larves est suivie pendant 2 mois et pendant ce temps les kystes sont conservés à une température de 25°C. Des comptages sont effectués régulièrement après 6-10-16-24-34-46-60 jours. A chaque comptage les larves écloses sont enlevées au moyen d'une pipette et un cm^3 de sécrétion radiculaire est ajouté. A la fin de l'essais le nombre de larves écloses est calculé en pourcentage du contenu total.

Cette technique se prête très bien pour l'étude approfondie des différents facteurs, ayant une influence quelconque sur l'effet nématicide et la distribution des fumigants, comme par exemple la température, la teneur en eau du sol, la quantité du fumigant appliquée, la distance entre les points d'injection, le type du sol, la profondeur d'application.

C. L'EXAMEN DE DIFFÉRENTES ESPÈCES DE SOLANUM QUANT À LEUR RÉSISTANCE

Les recherches sur la résistance de solanums sont exécutées de façon à ne laisser aucun doute concernant la formation de nouveaux kystes sur les racines. Les semis ou tubercules sont plantés en pots de terre cuite, remplis d'un demi litre de terre et artificiellement infectés par 400 kystes. Le nombre de kystes est mesuré d'une façon rapide au moyen d'un petit tube en verre dont le contenu correspond avec le volume de quelques 400 kystes. Les modifications naturelles qui se produisent dans la population des kystes pendant la période de croissance, sont étudiées au moyen d'une douzaine de pots de contrôle, infectés du même nombre de kystes et tenus dans les mêmes circonstances. L'examen de ces pots de contrôle a démontré que 15% du nombre de kystes ajoutés disparait, le contenu moyen par kyste restant pratiquement le même. Après la période de croissance chaque pot est renversé prudemment et une première sélection est effectuée basée sur la présence de kystes sur les racines superficielles. Les

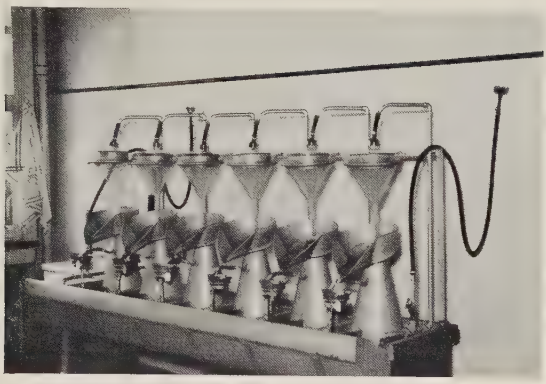

Fig. 1

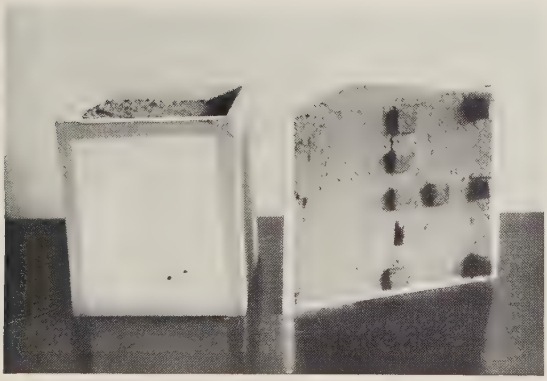

Fig. 2

Fig. 3

plantes infectées sont éliminées. Les plantes dont les racines ne portent pas de kystes et les plantes douteuses sont soumises à un examen minutieux. Le feuillage est enlevé, les graines et les tubercules sont rassemblés et conservés; le reste du pot est séché pour en récupérer les kystes. Si le nombre de kystes retrouvés dépasse largement celui des pots de contrôle, la plante est considérée comme infectée et éliminée à son tour. Si le nombre de kystes est du même ordre de grandeur ou légèrement supérieur à celui des pots de contrôle la plante est considérée comme possédant des caractères de résistance.

Le nombre de kystes retrouvés dans les pots à plantes résistantes était du même ordre de grandeur que celui des pots de contrôle, notamment 85% du nombre original; le contenu moyen par kyste au contraire était parfois tombé à un cinquième. Une très grande augmentation en nombre de kystes a été constatée dans les pots à plantes infectées. L'examen du contenu d'une dizaine de pots a démontré une multiplication de plus de dix fois; on y a retrouvé jusqu'à 4.600 kystes à contenu moyen doublé.

D. MÉTHODE POUR OBTENIR DE GRANDES QUANTITÉS DE KYSTES UNIFORMES ET DE LA SECRÉTION RADICULAIRE

Tous nos essais sont exécutés avec des kystes rigoureusement sélectionnés et uniformes en ce qui concerne le diamètre, le contenu et l'âge. Ils sont récoltés des racines d'une culture de pomme de terre très infestée, provenant d'un terrain sabloneux. Des espèces tardives conviennent le mieux, à cause de leur formation de racines plus forte et plus étendue. Une culture trop endommagée est moins utile parce que la quantité de petits kystes y est trop élevée et le contenu trop faible. Les plantes sont prélevées du sol au moment ou les kystes sont devenus légèrement bruns et que le sol est suffisamment sec. Les tubercules et la terre adhérente sont enlevés prudemment et les racines sont coupées au dessus d'un récipient, afin de prévenir une perte de kystes. Le matériel récolté de cette façon est étendu au laboratoire en couches minces et séché à l'air. Les racines sont régulièrement remuées et secouées pendant le séchage afin de détacher tous les kystes. Après séchage la terre et les kystes sont séparés des racines au moyen d'un tamis adéquat. Le résidu ainsi obtenu est sassé une deuxième fois au moyen d'un tamis à petites mailles retenant les kystes d'un diamètre minimum déterminé. Le résidu obtenu finalement est réduit à une petite fraction du matériel original; il contient des dizaines de milliers de kystes par kilo, dont on est certain qu'ils sont presque tous nouvelle-

ment formés. La récupération des kystes de ce résidu est effectuée au moyen de l'appareillage décrit. De cette façon de grandes quantités de kystes uniformes sont récoltées en peu de temps; des quantités de 20 gr et même plus sont facilement à atteindre par are.

La sécrétion radiculaire est obtenue d'une façon tout à fait différente de la méthode ordinaire: des tubercules sont mis à germer dans de verres à hyacinthes remplis d'eau (Pl. III, fig. 3). Les meilleurs résultats sont obtenus avec des tubercules de la variété „Bintje" dont les racines poussent très vite et très fortement. La sécrétion est récoltée deux fois par tubercule avec un intervalle de 2 à 3 semaines.

RÉSUMÉ

Les techniques développées et pratiquées relatives à l'examen de quelques problèmes sont traitées en rapport avec les recherches et l'étude des moyens de lutte contre le nématode doré de la pomme de terre.

Le système classique pour récupérer les kystes au moyen de la cruche de FENWICK a été modifié en ce sens, que le matériel flottant est décanté par un jet d'eau, dirigé verticalement dans la cruche au moyen d'un petit tuyau courbé, placé en dessous du collet. Ce système permet de rassembler en une seule opération et rapidement tout le résidu.

L'examen des produits chimiques, ayant manifesté des caractères nématicides est approfondi dans des essais en bacs. A cette fin les différents facteurs, pouvant avoir une influence sur l'effet nématicide et la distribution des fumigants, sont étudiés dans des bacs remplis de terre, dont les dimensions correspondent à la superficie couverte par un point d'injection sur le champ et à la profondeur de la couche arable. Le degré et les zones de l'effet nématicide sont controlés au moyen d'essais d'éclosion effectués sur des kystes qui ont été introduits à des points bien déterminés dans le sol.

Les recherches sur la résistance des espèces de Solanum sont exécutées d'une manière qui permet, en cas de doute concernant la présence de kystes sur les racines, de déterminer avec certitude le nombre de kystes nouvellement formés.

Le matériel biologique employé dans les essais est rigoureusement sélectionné et uniforme, de grandes quantités de kystes étant prélevées dans la laboratoire sur des racines de pommes de terre.

La sécrétion radiculaire est obtenue en laissant germer les tubercules de la variété „Bintje" dans des verres à hyacinthes, remplis d'eau.

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DISCUSSION

B. G. PETERS: On producing root secretion by growing tubers in hyacinth glasses is pure water used, or a nutrient solution?

LES AUTEURS: In this technique only ordinary tapwater is used.

J. H. SCHUURMANS STEKHOVEN: Can you give me any information about the phytotoxicity of the nematicides used on potatoes?

LES AUTEURS: Notre expérience concerne surtout le DD appliqué en terrain sablonneux. Nous n'avons pas observé de dégâts lorsque l'application du nématicide précédait la culture d'au moins un mois. Le traitement pendant la végétation, même à la faible dose de 2 l par a, s'est avéré très nuisible.

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NEW NAMES PROPOSED FOR THREE HOMONYMS *)

BY

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Dr. E. W. Price of the Zoological Department of the U.S. Bureau of Animal Industry, informed me that the species *Phanoderma elegans* Schuurmans Stekhoven 1950 (Mem. Inst. Roy. Sc. Nat. Belg., 2.s. (37) 220 pp.) is preoccupied by *Phanoderma elegans* Ditlevsen, confer: Schuurmans Stekhoven 1946, p. 14 and that the species *Symplocostoma longiseta* Schuurmans Stekhoven 1950 is already preoccupied by *Symplocostoma longiseta* Micoletzky H. 1930, p. 305-307 (Vidensk. Medd. Dansh. Naturk. Forening Kobenhavn, 87: 243-339. 1929-30), whereas *Anoncholaimus* Schuurmans Stekhoven 1950 is preoccupied by *Anoncholaimus* Cobb N.A. 1920 (Contributions to the Science of Nematology Cobb 9, pp. 220-221).

In their place the following names are proposed for these Homonyms:

a. *Phanoderma falcicauda* for *Phanoderma elegans* Schuurmans Stekhoven 1950.

b. *Symplocostoma obtusidens* for *Symplocostoma longiseta* Schuurmans Stekhoven 1950.

c. *Bradybucca* for *Anoncholaimus* Schuurmans Stekhoven 1950.

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POPULATION DECREASE OF HETERODERA ROSTOCHIENSIS AFTER DD TREATMENT OF THE SOIL

BY

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(T.N.O., Wageningen, Holland)

A. INTRODUCTION

A study was made of population decrease of *Heterodera rostochiensis* in absence of a hostplant after DD treatment of the soil.

At an infested trialfield with sandy soil, microplots were made by placing cement planks and pickets 55 cm deep in the soil. The corners between the planks were closed by means of cement to avoid migration of larvae from one plot to the other. The microplots were 1.08 cm x 1.08 cm (Pl. IV). They were sampled by means of eighty prods with a little borer to the depth of the tilth (20 cm).

At 14/4/1954 three plots were treated with 40 ml DD/m², three plots with 80 ml DD/m² and three plots remained untreated. DD was injected by means of pipet at sixteen places per plot, thirteen cm deep, soil temperature 10°C and moisture content of the soil was 0,24 of waterholding capacity. One month after treatment the plots were sampled again, in the same way as mentioned above, and planted with beans (*Phaseolus vulgaris*) to avoid blowing away of the soil.

Every three months samples were taken and treated as mentioned below.

B. MATERIAL AND METHODS

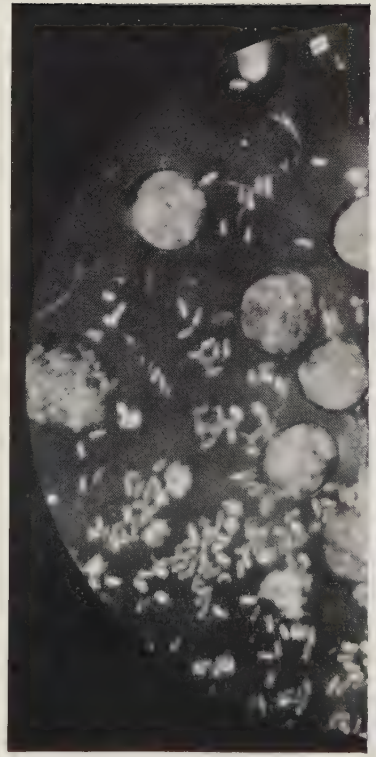
The day of taking the soil sample, the soil was spread on a paper and dried at room temperature. After the soil had dried well, it was mixed thoroughly and two samples (A and B) of about 300 grams of soil each were taken. (For the hatching experiment of the samples of 13/4/54 and 15/5/54 a larger sample was taken). The cysts in every sample were washed from the soil and removed.

The cysts of sample A were soaked in water for a week, after which potato root diffusate was added. Each week the hatched larvae were

1) Now nematologist, N. V. Philips Roxane, 's Graveland, Holland.

Microplots, used in these experiments (photo Plantenziektenkundige Dienst, Wageningen)

Fig. 1. Apparatus used in the homogenisor technique for estimating the contents of the cysts (photo: Plantenziektenkundige Dienst, Wageningen).



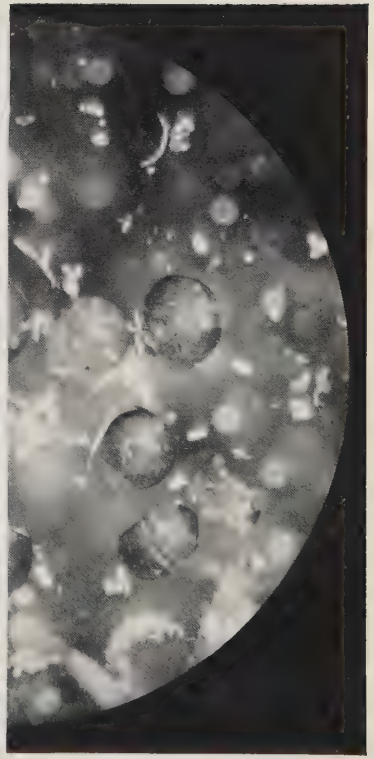


Fig. 2. Dissected cysts untreated. Fig. 3. Treated in homogenisor (photos: Plantenziektenkundige Dienst, Wageningen).

Fig. 1. Microphoto of a living larva within the eggshell.

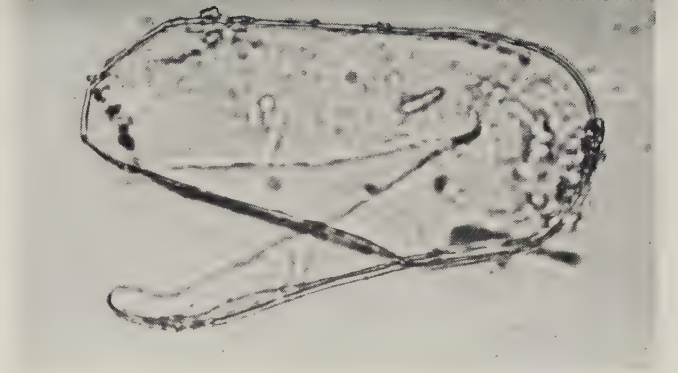


Fig. 2. Microphoto of eggshell after the larva has emerged.

Fig. 3. Microphoto of egg 10 months after soiltreatment with DD. The eggshell is closed and only the remains of the spear, →, can be found.

Fig 4. Microphoto of egg from soil treated with DD. The larva is just decomposing.

(photos: Plantenziektenkundige Dienst, Wageningen).

counted and removed and new potato root diffusate added. Hatching continued for 2½ month, after which the remaining (not hatched) eggs were estimated by means of the "homogeniser technique" (Bijloo 1954). The content of the cysts of sample B was also estimated by means of the "homogeniser technique".

In this technique cysts are placed on a damp perspex slide and dissected with a Hagedorn-needle. They are then placed in a bottle of a CEKA-homogeniser with about 25 ml of water. The apparatus is spun for three minutes at about 8.000 R.P.M. The material is thrown outwards onto the glass wall of the bottle and with every rotation thrown against the ridges of the bottle. The cysts content is shaken from the cyst walls, which appeared to be quite empty after treatment (Pl. V). The suspension was then diluted to 200 ml and 4 samples of 1 ml were counted.

In these samples a differentiation was made between dead eggs, brown (dead) eggs, living eggs, empty eggs and living larvae, which equal total content of the cysts.

Just after treatment most of the eggs from the cysts of the fumigated plots seemed to be as clear as from the untreated ones, and there was no visible difference. By touching egg after egg with a micro-needle, the larvae from the living eggs jumped out of the egg and after some seconds showed a tendency to bend a little. From the clear dead eggs the larvae jumped out after touching, but rested stretched. These eggs proved to be not hatchable, according to the results of the hatching experiment of the sample A. Seven months after treatment the larvae from the dead eggs came out after touching, but rested more or less in a folded up position.

C. RESULTS

The means of the countings of the three repetitions are calculated per cyst and can be found in the first part of table I (column 3, 4, 5, 6, 7 and 8). These numbers are also calculated as percentages of the total contents.

The second part of this table (column 10, 11, 12, 13, 14 and 15) gives the results of the hatching experiments with the cysts of samples A, also calculated per cyst. Column 16 shows the percentage of hatched eggs calculated from the total number of full eggs per cyst. Because of interfering effects of fungi in the laboratory-test at the end of the hatching experiments it was not always possible to get a good impression of the number of empty eggs after hatching. There-

fore in column 17 the total content per cyst of the hatching experiment is calculated by adding the number of empty eggs in column 7 (calculated from the cyst of sample B), to the number of hatched larvae per cyst in column 13 and the number of not hatched eggs per cyst in column 15 (calculated of sample A).

In a comparison between the figures in column 3 and those in column 17 we see the total contents of cysts agree well. Therefore the percentage of hatched larvae of total contents calculated in column 18 can be compared with data from cysts of sample B.

From the untreated plots is seen that from the 172,2 (207,3—35,1) full eggs per cyst at the beginning of the experiment only 105,5 eggs remain on the 15/8/54, which means that ± 39% of the full eggs have disappeared. This figure is somewhat lower than Dr. FENWICK's (1950), and Dr. OOSTENBRINK's (1952), who suggest a remaining number of about 50% of the content of full eggs.

But when we study the living eggs (and they cause the disease) it is seen that from 115,3 living eggs at the beginning of the experiment only 34,4 living eggs remain. This means a reduction of about 70%. According to the increasing number of empty eggs during this period (from 35,1 till 97,1 per cyst) it seems that about 64% of the living larvae have emerged, whilst some of the eggs died (see increasing number of dead and brown eggs). At the same time in tests conducted in the laboratory between 28/4/54 and 9/7/54 100,1 of the 115,3 living eggs could be hatched (86%).

The decline of the population can easily be seen in fig. 1. In this diagram numbers are given as percentage of the total contents to eliminate seasonal deviations and individual influences. It can be seen easily that the decreasing number of living eggs is greatly compensated by the increasing number of empty eggs. At the same time it appears that from 15/8/54 till 15/2/55 the number of living eggs does not change: the cysts are in dormancy. After 15/2/55 dormancy is over and emergency starts again. It can also be noted that there is a small decrease of dead eggs, which seems to be compensated by an equally small increase of the empty eggs. At the end of this paper we shall show how this can be explained.

From the hatching experiments of the samples A, percentages emerged larvae, in presence of potato root diffusate, are calculated. (Table 1, column 18). These figures are graphed. Though hatching took place between the two dates, the result is placed at the first date

because it had to be compared with those of the sample B of the same time.

It may now be seen that the hatching curve remains always below that of the living eggs. This curve also shows dormancy, with its minimum in the period between 15/8/54 till 15/11/54. In the field dormancy continued till about 15/2/55 in the laboratory more larvae emerged between 15/11/54 and 15/2/55 than in the previous period.

From the treatment graphs it is seen that one month after treat-

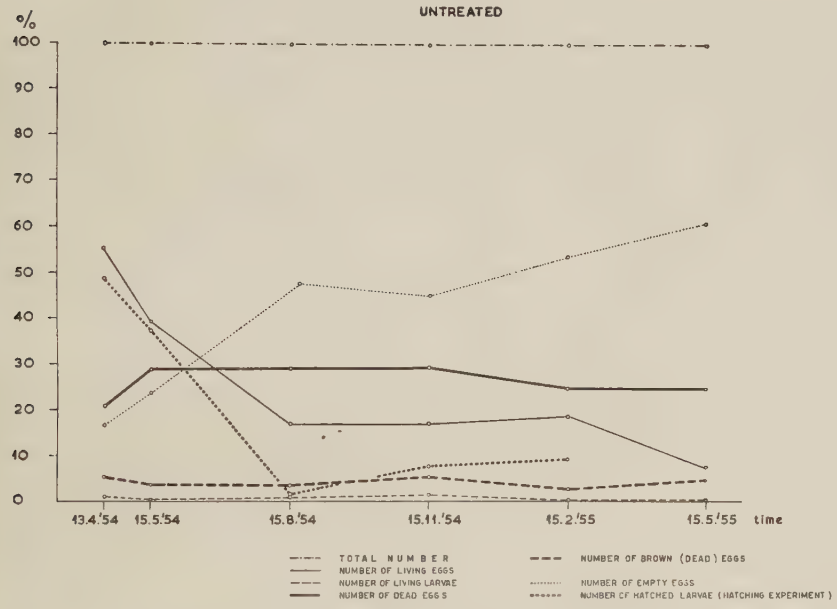


Fig. 1. Average composition of cysts contents in absence of the host-plant (control). Each point of the curve represents the average content per cyst in % of the total content as a mean of about 500 cysts.

ment it is found, there is a correlation between percentage of dead eggs increasing and living eggs decreasing. There was little difference in kill between injections with 40 ml DD/m^2 and 80 ml DD/m^2.

One month after treatment the controlling effect of DD had stopped since the percentage of killed eggs remained constant or decreased. It may therefore be assumed that DD had no "after effects". This is also seen from the decrease of the percentage of living eggs after the 15/4/54, which is due to emergence of living larvae (hatching curve).

On the other hand it appears that there is a correlation between the

Average composition of the cyst contents in absence of a hostpi

Time Treatment Average composition of the cyst contents (homogenisor technic

1 2 3 4 5 6 7 8

Time	Treatment		Total contents	Dead eggs	Brown (dead) eggs	Living eggs	Empty eggs	Larvae
I	0		207.3	43,3	11,1	115,3	35,1	2,5
13/4/54		%	100	20,9	5,4	55,6	16,9	1,2
before								
treat-	40		208,9	35,5	12,6	118,1	39,9	2,9
ment		%	100	17,0	6,1	56,5	19,1	1,4
	80		213,8	43,6	9,6	120,9	37,2	2,5
		%	100	20,4	4,5	56,6	17,4	1,2
II	0		203,8	58,6	7,8	80,6	48,8	0,6
15/5/54		%	100	28,8	3,8	39,6	23,9	0,3
	40		218,5	181,4	8,9	10,2	18,4	0
		%	100	83,0	4,1	4,7	8,4	0
	80		234,2	188,3	9,0	5,9	30,7	0,3
		%	100	80,4	3,8	2,5	13,1	0,1
III	0		202,6	59,0	7,6	34,4	97,1	2,0
15/8/54		%	100	29,1	3,8	17,0	47,9	1,0
	40		235,4	178,5	7,2	5,5	44,3	0
		%	100	75,8	3,1	2,3	18,8	0
	80		208,2	171,1	4,7	1,1	31,4	0
		%	100	82,2	2,3	0,5	15,1	0
IV	0		169,3	50,0	9,7	29,2	76,1	2,9
15/11/54		%	100	29,5	5,7	17,2	45,0	1,7
	40		180,4	126,7	6,6	2,7	42,5	2,0
		%	100	70,2	3,7	1,5	24,0	1,1
	80		165,5	128,7	7,0	0,6	28,4	2,9
		%	100	77,8	4,2	0,4	17,2	1,8
V	0		190,5	46,8	5,6	36,8	100,8	1
15/2/55		%	100	24,6	3	19,3	52,9	0,5
	40		182,2	132,1	8,5	2,2	44,4	1,8
		%	100	72,5	4,7	1,2	24,4	1
	80		193,1	130,7	7,9	0,3	48,3	0,3
		%	100	67,7	4,1	0,2	25,0	0,2
VI	0		213,4	53	10,2	16,9	130,0	1,8
15/5/55		%	100	24,8	4,8	7,9	60,9	0,8
	40		227,2	142,4	8,0	2,2	72,9	1,6
		%	100	62,7	3,5	1,0	32,1	0,7
	80		204,9	125,3	5,4	0,6	73,4	0,6
		%	100	61,2	2,9	0,3	35,8	0,3

I

ter soil treatment with 40 ml. and 80 ml. DD/m² (O = Untreated)

Hatching experiment

10	11	12	13	14	15	16	17	18
Time of hatching	Number of cysts	Hatched larvae	Hatched larvae per cyst	Not hatched eggs	Not hatched eggs per cyst	% Hatched of 12 + 14	Total contents (7 + 13 + 15)	% Hatched larvae of the total contents (from 13 and 17)
from 8/4/54 till 9/7/54	1226	122698	100,1	85750	69,9	58,9	205,1	48,8
	1317	121526	92,3	79350	60,3	60,5	192,5	48,0
	1174	131698	112,2	71100	60,6	64,9	210,0	53,4
from 4/5/54 till 9/8/54	742	57476	77,5	60300	81,3	48,8	207,6	37,3
	678	11366	16,8	113950	168,1	9,1	203,3	8,3
	680	3302	4,9	123334	181,4	2,6	217,0	2,3
from 2/9/54 till 0/11/54	552	1906	3,4	52550	95,2	3,5	195,7	1,7
	508	320	0,6	72910	145,5	0,4	190,4	0,3
	571	289	0,5	83710	146,6	0,3	178,5	0,3
from 9/11/54 till 4/2/55	571	7412	13,0	42750	74,9	14,8	164,0	7,9
	514	697	1,4	74050	144,1	0,9	188,0	0,7
	593	595	1,0	89000	150,1	0,7	179,5	0,6
from 5/2/55 till 8/4/55	455	7781	17,1	29300	64,4	21,0	182,3	9,4
	591	1286	2,2	80100	135,3	1,6	181,9	1,2
	560	787	1,4	78200	137,7	1,0	187,4	0,7

percentage of dead eggs decreasing and the percentage of "empty" eggs increasing. Brown eggs remain constant.

When we look at table 1 and at the graphs of fig. 2 and 3 it appears that there are hardly any living larvae in the cysts and the hatching curve remains below that of the living eggs. (Only one point of the hatching curve in fig. 2 is above the curve of the living eggs. The others lie below this curve. It is suggested that this is thought to be due to a sampling error). This means that only a very small number of eggs are

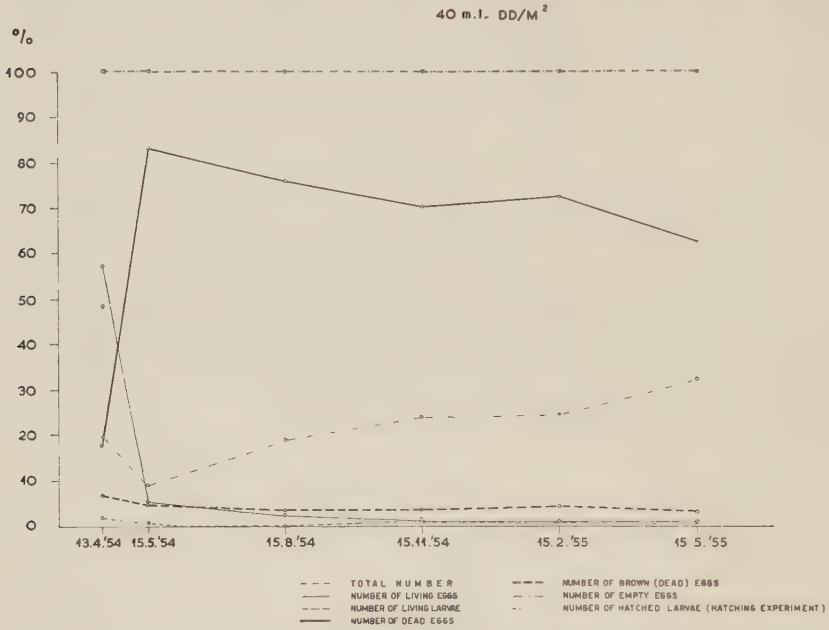


Fig. 2. Average composition of cysts contents in absence of the host-plant after soil-treatment with 40 m.l. DD/m². Each point of the curve represents the average content per cyst in % of the total content as a mean of about 500 cysts.

hatchable even ten months after treatment. It is not possible that all "empty" eggs resulted from larval emergence. We must therefore look elsewhere for an explanation.

When examining microscopically with a magnification of 500, this solution was found. Most of the "empty" eggs from treated plots proved to be not empty at all. In the first place, the eggshells were closed in contrary to those of the hatched eggs from untreated plots, which were open. In the second place, within the eggshells of treated cysts, larval remains were detected, such as rests of the spear or other body-parts.

In Pl. VI, fig. 1 a microphoto of a living larva within the eggshell is shown. Pl. VI, fig. 2 shows the eggshell after the larva has emerged; this shell is opened. Pl. VI, fig. 3 shows an egg 10 months after soil treatment. This eggshell is closed and only the remains of the spear can be found.

It becomes therefore obvious that the larvae could not emerge. Pl. VI, fig. 4 shows an egg from the treated plots at the moment the larva decomposes. Larval remains are found within the closed eggshell.

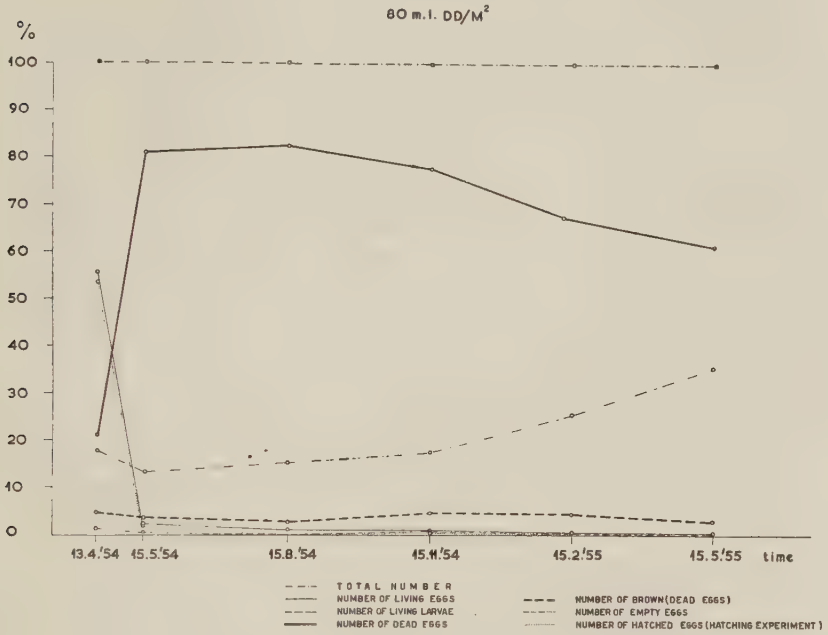


Fig. 3. Average composition of cysts contents in absence of the host-plant after soil-treatment with 80 m.l. DD/m². Each point of the curve represents the average content per cyst in % of the total content as a mean of about 500 cysts.

When studying these eggs with a binocular-microscope at a magnification of 40 times these remains within the eggs are mostly too small to be seen and the eggs therefore classified as empty. This, we feel, could be the reason for Dr. GRAINGER's conclusion (1954 a, b) that one of the "after-effects" of DD is that "it can stimulate eggs to hatch in the absence of a potato crop". But it should be stressed that they are not hatched at all.

Because of this and the fact that only very few (survivors) of the eggs of the treated plots are hatchable (see table 1 and fig. 2 and 3)

it is concluded that DD does not stimulate eggs to hatch, but kills the eggs. After the eggs are killed they remain clear for some months, often giving the impression of being alive. After a longer time however the larvae within the eggs decompose but do not become brown. The eggshells now seem to be empty, but they are not opened and the remains of the larvae prove that they have not emerged.

SUMMARY

Using micro-plots on an infested trialfield in sandy soil a study was made of population decrease of *Heterodera rostochiensis* in the absence of a hostplant. This experiment was made with three replications with an application of 0 ml, 40 ml and 80 ml DD/m² injected at 13 cm depth. Prevailing soil temperature was 10° C, whilst watercontent of the soil was 0,24 of waterholding capacity with organic matter 2,5%. Just before treatment, as well as one month after treatment and further every three months later a soil sample was taken by means of 80 prods with a little borer to the depth of the tilth.

After drying at room temperature, and one day after sampling two samples A and B of about 300 gram of the soil were taken, cysts were washed out. The cysts of sample A were after soaking in water exposed to potato root diffusate. 2½ month later the unhatched eggs were estimated by means of the "homogeniser technique" and percentages of hatched larvae calculated. By means of the "homogeniser technique" content of the cysts of sample B were estimated and classified: dead eggs, brown (dead) eggs, living eggs, empty eggs and living larvae, which represent the total content of the cysts. These figures were calculated as percentage of the total content and graphed. Each point presented in the diagram, was calculated from app. 500 cysts. From the untreated plots it was seen that about 39% of the full eggs had emerged in the soil from cysts between 13/4/54. and 15/8/54. According to living eggs only, there was a reduction of 70% from which 64% had emerged. (They were calculated from the increasing number of empty eggs). In the hatching experiment of sample A 86% of living larvae were hatched. From 15/8/54 till 15/2/55 the cysts were dormant and only very few larvae were hatchable; after the 15/2/55 emergence restarted. From the treated plots (40 ml and 80 ml DD/m²) it was seen, that one month after treatment DD had killed a large number of the eggs and larvae but had no further aftereffect on the cysts. Some of the eggs of these cysts of treated plots proved to be hatchable (sample B), but always less than the number of living eggs according to sample A.

Seven months after treatment the number of empty eggs within cysts from the treated plots began to increase whilst the number of dead eggs began to decrease.

Moreover the hatching experiment of sample A proved that these eggs were not hatchable. When studying microscopically with a magnification of 500 these empty eggs of treated plots proved to be unhatched; killed larvae proved to be decomposed within the eggshells, according to deposits including rests of the spear etc.

Because of this it is stated that DD does not hatch larvae from the cyst in absence of a potato crop, but kills the larvae within the eggs. After a long period the larvae decompose within the eggshell. The eggshells are closed contrarily to those of the hatched eggs, and remains of the larvae (spear etc.) may only be seen at high magnification.

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DISCUSSION

J. GRAINGER: Results from my similar work at Auchincruive, Scotland, agree in showing a wastage of cyst-contents in the absence of potatoes, following the application of D.D.

It would also appear that a) a killing of larvae occurs *outside* the egg but still within the cyst, during the first year after injection, and b) hatching of eggs into larvae may occur, but mostly in the second and third year after injection.

Action a) may be parallel to that described by Mr. Bijloo and Miss Boogaers, and it would be good to see if they could still obtain b) in subsequent years.

J. D. BIJLOO: We are sure that the number of "empty eggs" will increase the following years, for this is in good agreement with the results in the diagrams of fig. 2 and fig. 3. These "empty eggs" are a result of two different mechanisms:

1) A further decomposing of killed larvae within the eggs as shown in Pl. VI, fig. 3 and 4.

2) Emergence of larvae from eggs that were not killed by the D.D. This emergence can be seen in the diagram of fig. 1 (decreasing number of living eggs) but also in the diagrams of figs. 2 and 3 (decreasing number of living eggs). This is a normal hatch caused by soil leachings and has no relation to the D.D.-treatment.

J. H. SCHUURMANS STEKHOVEN: asked if also experiments "in vitro" were done either with presoaked or not presoaked larvae. Is there a nematolysis of the larvae within the cysts? Further information is requested about the criterion whether the larvae were living or dead.

J. D. BIJLOO: The experiments are all made in vivo at microplots. One month after D.D.-treatment of the soil the only criterion was that killed larvae jumped out of the eggshell after touching with a needle, and remained stretched. At the same time living larvae jumped out of the eggshell after touching, and after some seconds showed a tendency to bow.

The results obtained in this way were in good agree with those of the hatching experiments of cysts of the same plots (figs. 1, 2 & 3). Some months after the D.D.-treatment of the soil, the killed larvae remained kinked after freeing them from the eggshell, whereas the living ones jumped out as mentioned above.

H. R. WALLACE: Experiments on the hatching of sugar-beet-eelworm in sand at optimum moisture and aeration gives a high rate of emergence. If, however,

the vulval and oral openings of the cysts are sealed, the egg-hatch within the cyst is very low and few free larvae are found within the cyst. This would suggest that the presence of free larvae within the cyst inhibits further egg-hatch.

With regard to the question of kinking as a criterion of death I should like to make an observation. When eggs are placed in hypertonic solutions of sugar (sucrose) the unhatched larvae shrink and kink, but they are still capable of hatching in water when the larvae swell. This suggests that kinked larvae are not necessarily dead.

J. D. Bijloo: I agree with Mr. Wallace that when placing eggs in hypertonic solutions of sugar etc., the unhatched larvae can shrink and kink. But this is a very abnormal situation which never will occur with cysts in the soil at normal fields.

D. W. Fenwick: asked for information on the time allowed by Mr. Bijloo for this cysts to soak prior to dissection. He pointed out that in his experience soaking time had an influence on the number of larvae emerging on dissection. Soaking increased this figure: soaking also influenced the degree of kinking of the larvae, dissection of dried cysts could result in the majority of the liberated larvae being kinked—hence kill estimated on this criterion might be deceptive.

J. D. Bijloo: To avoid this phenomenon the cysts were washed from the soil as soon as it was dry enough: the cysts were then examined before the eggs were totally dry.

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REPRINT WEST OF SCOTLAND AGRICULTURAL COLLEGE

PROGRESS IN SOIL MIXING FOR NEMATODE CONTROL

BY

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Cysts of *Heterodera rostochiensis* have been found at depths from 28 to 36 in. (71 to 91 cm.) in potato land in west Scotland (O'Brien and Prentice 1932). The vast majority, however, are present in the top 8 in. (20.3 cm.) of soil. Now the top 8 in. from one acre would form a large heap 25 by 10 by 4 yds. A similar depth from a hectare would make a heap 21 by 15 by 6 metres. Any effective control must reach every particle of such great masses. Volatile materials like D.D. can spread as vapour, but certain less expensive non-volatile materials such as mercury compounds, can control eelworm diseases, but must be distributed mechanically to all parts of the soil mass before they are effective.

MERCURY COMPOUNDS FOR THE CONTROL OF HETERODERA ROSTOCHIENSIS

Mercuric chloride was found by Johnson (1936) to reduce the infestation of potatoes by root eelworm. He used 194 to 242 lb of the solid material per acre (219 to 272 Kg. per hectare), lightly raked into the surface three weeks before planting the crop. The treatment was uneconomic and lowered the potato yield, but reduced eelworm infestation during the growth of a crop by 16 to 36%. Larval eelworms within the eggs were not killed by the field treatment, though in the laboratory eggs were prevented from hatching by immersion for 2 min. in 0.1% mercuric chloride solution.

The writer (Grainger, 1951) later showed that very much smaller amounts of mercuric chloride (equivalent to 7.5 lb. per acre or 8.4 Kg. per hectare) were effective if mixed *thoroughly* with the soil shortly before the potatoes were planted. Initial work was necessarily confined to pot experiments, for the thorough mixing had to be done by hand methods where liquids were finely sprayed, or dusts finely blown, upon the soil as it was mixed upon a concrete floor. The quantities of material and soil were, however, carefully weighed in order that

approximate rates per acre could be visualised. Table 1 collects a number of results, from which the following conclusions can be drawn:

1. The best control (82 to 87%) was given by liquid applications of the smallest mercury equivalent used (5.4 lb. per acre or 6.1 Kg. per hectare), and this amount would cost less than a quarter the price of 200 lb. of D.D. Doubling or quadrupling the amount of solution did not increase the degree of eelworm control (1, 2 & 3, table 1), which seems to vindicate the adequacy of mixing. The application, however, required too large a quantity of water (750 gall. per acre), so dusts, and more concentrated solutions were tried.

2. Thoroughly mixed dusts were, for comparable mercury equivalents, less effective than a thoroughly mixed dilute liquid, and, unlike the liquid, increasing the amount of dust improved the degree of control (compare 6-8, 9-11 with 1-3 in table I). This seems to suggest an uneven distribution of mercury material within the dust (see below). The smallest amount of dust used (6 in table I) was equivalent to about 10 cwt. per acre (1,250 Kg. per hectare), and no difficulty was experienced in mixing this quantity. Grey powder (dust B in table I) has finely-divided mercury in the elemental form, and this still brought about a useful degree of control.

3. Difficulty was experienced in mixing adequately a small volume of relatively concentrated mercuric chloride solution (4, table I), and the amount of control obtained (only 13%) reflected this difficulty. Since the mercury equivalent per acre and the chemical form were the same as in 1, table I, which gave 82 to 87% control, the difference in performance must have been due to the difference in mixing, and a comparison of these differences should lead to a specification of what is meant by adequate mixing. Doubling the amount of concentrated solution more than doubled the percentage control (compare 5 with 4, table I) thus emphasizing the inadequacy of distribution.

4. The effect of one application was not necessarily confined to its immediate crop; there was often a residual effect on a subsequent crop (1, 2, 3, 9, 10 and 11 for 1953 in table I).

Mercury compounds can apparently control parasites by direct destruction, when used against seed-borne fungi or as dips for bulbs or corms. Oostenbrink & Stofmeel (1953) for instance, demonstrated a killing of eggs within cysts of *H. rostochiensis* adhering to gladiolus corms, by means of a mercurial dip. When mercury compounds are added to the soil mass, however, they are, as shown by Booer (1944), ultimately reduced to elemental metallic mercury, which

TABLE I

Pot Experiments with Mercury Compounds

Equivalent rate of application

Material		*Mercury equivalent* lb. per acre	*Mercury equivalent* Kg. per hectare	Mercury compound lb. per acre	Mercury compound Kg. per hectare	Water gall. per acre	Water l. per hectare	Cysts with contents per g. of soil [1] *1949*	*1950*	*1953* [2]	Percentage control *1949*	*1950*	*1953* [2]
Mercuric Chloride	1.	5.4	6.1	7.5	8.4	750	8,620	0.5^{s}	0.6^{s}	$1.6^{(s)}$	87	82	41
Solution of	2.	10.8	12.2	15.0	16.8	1,500	17,240	0.6^{s}	0.9^{s}	1.4^{s}	84	72	48
1 in 1,000	3.	21.6	24.4	30.0	33.6	3,000	34,480	0.4^{s}	0.8^{s}	0.5^{s}	89	75	82
with water	Control	0	0	0	0	750	8,620	3.7	3.2	2.7			
Mercuric Chloride	4.	5.4	6.1	7.5	8.4	150	1,724	1.4^{n}	—	—	13		
Solution of	Control	0	0	0	0	150	1,724	1.6	—	—			
1 in 200	5.	10.8	12.2	15.0	16.8	300	3,448	2.1^{n}	—	—	28		
with water	Control	0	0	0	0	300	3,448	2.9	—	—			
						Dust as applied lb. per acre	Dust as applied Kg. per hectare						
A. *Tolyl Mercury*	6.	10.9	12.2	16.5	18.6	1,101	1,242	2.6^{n}	—	—	30		
Acetate and Ethyl	7.	21.8	24.4	33.0	37.2	2,202	2,484	$1.9^{(s)}$	—	—	49		
Mercury Chloride	8.	32.7	36.6	49.5	55.8	3,301	3,726	1.5^{s}	—	—	60		
Dust containing 1.5% w/w of compounds	Control	0	0	0	0	0	0	3.7	—	—			
								1951	*1952*	*1953* [2]	*1951*	*1952*	*1953* [2]
B. *Grey Powder*	9.	5.9	6.5	17.6	19.9	1,762	1,990	1.9^{s}	1.8^{n}	3.3^{n}	25	12	0
(finely dispersed	10.	11.8	13.0	35.2	39.8	3,524	3,980	1.6^{s}	1.2^{n}	2.8^{n}	36	39	0
elemental mercury)	11	17.7	19.5	52.8	59.7	5.286	5,970	1.5^{s}	0.7^{s}	$1.7^{(s)}$	41	65	39
Dust containing 0.33% Hg. w/v.	Control	0	0	0	0	0	0	2.5	2.0	2.8			

1) Measured after the growth of potatoes: the original infestation of all soil used varied between 0.4 and 0.9 cysts with contents per gram.

2) Figures for 1953 are residual values from the previous treatment, i.e. 1950 for mercuric chloride solution and 1952 for grey powder. Potatoes were grown in 1953 for both experiments, but none in 1951 or 1952 for the mercuric chloride experiment.

^{s}, $^{(s)}$ and ^{n} Figures for eelworm populations are the averages of 5 pots, and those marked ^{s} are, statistically, significantly different from their respective controls. Those marked $^{(s)}$ are barely significant, and those marked ^{n} are not significant, all at the 5% levels.

has no direct parasite-killing action. The same author later suggested (1951) that their known action in controlling parasites arose from the effect of mercury vapour in retarding the activity of the parasite more than that of the host, thus disturbing the relationships necessary for disease to occur. That the final distribution appears to be by vapour is most fortunate, for the treatment which gave the best degree of control (1, table I) only provided one part by weight of mercury to 414,815 parts by weight of soil. The present demonstration that finely divided metallic mercury (in grey powder, dust B of table I) can control *H. rostochiensis,* supports the first part of Booer's work, while his later suggestion is in line with JOHNSON's (1936) statement that soil applications of mercuric chloride did not kill eggs of the potato root eelworm, whilst treatment in the laboratory prevented them from hatching.

Mercury reduced from 1 in 1,000 mercuric chloride solution mixed very intimately with the soil would presumably be in an extremely fine state of subdivision. The globule size is not known with any certainty, but preliminary indications from old and somewhat reduced mercuric chloride solution suggest that it would not be more than 1 μ in diameter, and may indeed be much less. This compares with 3 to 15 μ in grey powder (dust B, table I) and with aggregates (of the compounds) of 40 to 80 μ in the organic mercurial seed-dressing dust (A in table I). The percentages of control obtained are in inverse order to these relative particle sizes, and such a working hypothesis would explain why a dust can apparently be mixed thoroughly and yet give a relatively poor control. The large aggregates of dust A, for instance, contain about 65% of mercury w/w, and when reduced in the soil would bring large local concentrations of that element, giving unequal distribution. Thus inadequate mixing can apparently arise either from unsuitable physical conditions within the material, or from an unsuitable degree of incorporation of that material with the soil. The present investigation, however, was planned primarily to study the distribution of non-volatile compounds in soil and to mechanize a process for their adequate mixing. When radioactive materials became available they provided a neat and exact method for such an investigation. They could be used in place of the mercury and their distribution could easily be measured.

RADIOACTIVE "TRACERS"

The radioactive isotope Iodine^{131} was chosen for the present investigation; it has a half life of 8 days, which gives sufficient time

for effective work, without raising difficulties of disposal after the investigation is finished, for treated soil loses its radioactivity after 80 days — ten times the half life. All work was done in a special hut and enclosure with sump drainage within the enclosure. Each experiment lasted over 3 to 5 days, and the total amount of radiation reaching each worker was a good deal less than that from a radioluminous wristlet watch.

Iodine^{131} is supplied in the form of a solution of sodium sulphite. The amount of liquid providing 10 millicuries of radiation was generally diluted to one litre with water which was then sprayed evenly on to a plot of soil about 1 yard or 1 metre square, by means of a fine paint-spray gun. Placings at lower depths were sometimes used and were made experimentally by removing soil to the depth required, spraying the surface thus exposed with the I^{131} solution and then replacing the soil. The amount of radioactive solution mentioned above was sufficient to give measurable activity in all parts of the plot size used, down to a depth of 9 in. (22.9 cm.). In many cases this was tested by removing all the layers of soil from the full depth of a treated plot, mixing them thoroughly by 'hand' and then taking randomised readings at all parts of the mass. No part was then found to be without measurable activity.

Several kinds of mixing machine were considered, from extant rotary cultivators to various new ideas, but after consultation with the Scottish Machinery Testing Station, Howden, Midlothian, the new ideas were seen to have serious shortcomings. Most of the work was ultimately done with a Rotary Hoe of standard type with solid tines (Pl. VII, fig. 1), and a Rototiller with spring tines. These rotary cultivators worked at a speed of about 0.75 m.p.h. (1.2 k.p.h.) with the low gear used, with rotors revolving at about 150 r.p.m. A Roto-spade (Pl. VII, fig. 1) was also used, in which two stirrers of interrupted helical pattern revolved at about 60 r.p.m. in opposite directions vertically in the soil, the machine moving very slowly at 0.17 m.p.h. (0.27 k.p.h.). When two machines were used for a combined treatment, the second operation was made at right angles to the first. Machines were thoroughly hosed down with water immediately after use, to remove loose radioactive material, and if then still radioactive they were kept in quarantine within the special hut or enclosure for 80 days.

After the mixing device or a combination of machines had been run over the treated plot, the distribution of I^{131} which it brought about was assessed by means of a Geiger probe tube and monitor. The probe

tube was enclosed within a lead casing two inches (5 cm.) thick through which only a very small "window" 2 in. x ¾ in. (5 x 1.9 cm.) allowed access of radiative rays from the soil. The "window" faced vertically downwards through the lead casing when it was placed on the soil as shown in Pl. VII, fig. 2. This restriction of ingress of radiation increased the accuracy of the measurement greatly, though it made the work very laborious, since the apparatus weighed 1 cwt. (51 Kg.).

The lead-sheathed Geiger probe tube was moved over the surface of the treated plot, readings being taken at intervals of 1 in. (2.5 cm.) and when a series had been taken across the plot, the probe tube was moved over two inches (5 cm.) and another line of readings taken, again at intervals of 1 in., in the reverse direction. The process was repeated until the distribution of I^{131} over the whole surface or an adequate part of it had been "mapped".

A layer of soil 1 in. deep was then removed from the treated plot and the process of mapping repeated on the surface thus exposed. Further layers of soil 1 in. deep were subsequently removed and the exposed surfaces mapped until the required depth of 9 in. (22.9 cm.) was reached or until no further radiation could be detected.

There is always a certain amount of extraneous "background" reading, and the amount of this was measured at frequent intervals through the whole series of measurements. The number of "background" readings was generally at least half the number of those made on treated soil, and the appropriate average background reading was subtracted from any particular reading from radioactive soil, or from the average of a group of readings.

This method of mapping the soil in layers is considered to be superior to a possible alternative of cutting the treated mass into small cubes, the radiation of which could then be estimated separately. It is very much easier in practice to remove a layer of soil 1 in. thick from a fairly compact mass than to cut it accurately into small blocks. Stones interfere greatly with the latter, but not so much with the former process. Estimation *in situ*, however, requires a special form of interpretation in depth, for if a mass of soil were treated uniformly, the upper layers would give higher readings than the lower layers, because radioactive radiation can penetrate through several inches of soil. The upper layers therefore have not only their own innate radioactivity but also receive an additional amount from the layers below. This is a difficulty, however, which is overcome by arithmetic, once the "mean of uniform mix" is known. A considerable amount of work

Fig. 1. (left) The solid tine Rotary Hoe used for mixing; the Rototiller was similar but had spring tines. — (right) The Roto-spade; the machine is driven electrically and in use the interrupted helical paddles work vertically in the soil.

Fig. 2. (left): Taking readings with the radiation monitor. — (right) Moving the lead-sheathed Geiger probe tube over a treated plot. Two strips of wood marked in inches are laid down on each side. The position of the ingress "window" is marked approximately mid-way along the sheath.

has been done to estimate this mean, with results shown in table II. The mean varies for different working depths in the soil, and in the results which follow, all the diagrams have been corrected for this mean, where it applies.

TABLE II

The mean of uniform mix

Percentages of the arithmetical mean for an even distribution (± 20%) of I^{131}. (for radiation which penetrates 3 to 4 in. (7.5 to 10 cm.) into the soil)

Position of readings, downwards in soil.			Working depth in soil									
		Cm.	25	22.5	20.0	17.5	15.0	12.5	10.0	7.5	5.0	2.5
Cm.	In.	In.	10	9	8	7	6	5	4	3	2	1
Surface	Surface		126	126	126	126	126	126	125	118	110	100
2.5	1		123	122	122	122	120	116	109	102	90	0
5.0	2		121	119	118	115	111	102	92	82	0	
7.5	3		117	115	111	106	97	87	73	0		
10.0	4		113	108	102	93	82	68	0			
12.5	5		105	98	89	78	65	0				
15.0	6		95	86	75	62	0					
17.5	7		80	73	58	0						
20.0	8		66	56	0							
22.5	9		54	0								
25.0	10		0									

As a reading is taken on the surface, the arithmetical mean is that of a series of readings or average readings at 1 inch intervals to the working depth in inches *minus one.*

THE DEGREE OF MIXING REQUIRED FOR EELWORM CONTROL

The first work with radioactive iodine was devoted to an investigation of mixing with sufficient and insufficient amounts of liquid associated with the successful (1, table I) and unsuccessful (4, table I) control of *Heterodera rostochiensis* by mercuric chloride solution as mentioned above. It was found that an adequate mixing similar to that which gave 87% control of the build-up of the eelworm population did not vary more than ± 20% of the mean value, whereas the attempted mixing of an inadequate amount of liquid, though of equal mercury equivalent, but giving only 13% of eelworm control, varied widely outside ± 20% of the mean (fig. 1). Adequate mixing is therefore attained when the distribution at all places within the volume required lies within ± 20% of the desired mean value, except for a possible tolerance of about 5% within ± 30% (fig. 2). The tolerance is inferred from the data for adequate mixing in fig. 1. Results from table I suggested that even large overdoses of mercury at least did not diminish the degree of eelworm control; they even enhanced it

when dusts of large particle size were used, or when the distribution was otherwise inadequate. This might suggest that overdoses were not important in this specification. It is necessary, however, to keep economic considerations firmly in view, and overdoses are wasteful of resources, so the specification was used as stated, except that a tolerance of 5% within + 30% was regarded as more acceptable than one within — 30%.

PERFORMANCE OF EXISTING MACHINES

None of the machines mentioned above gave an adequate mix from a surface application alone. The most important lack was usually that

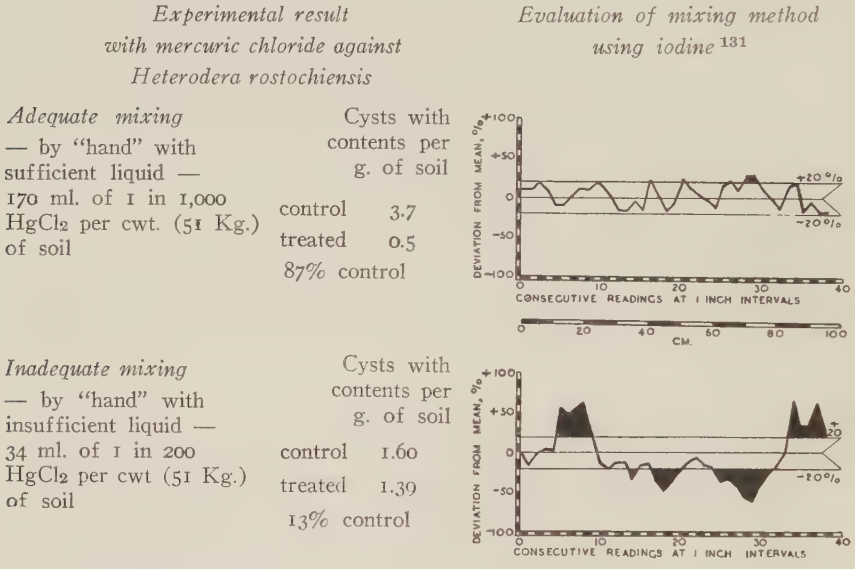

Fig. 1. The relation between performance in control of *Heterodera rostochiensis* and the intimacy of mixing.

of a sufficient distribution downwards into the soil. Fig. 3 shows a number of unsuitable distributions in depth, not only from surface placings but also from certain others. The Roto-spade is interesting in that, though it does not give an acceptable distribution, it nevertheless brings a certain amount of treated material to the full depth of working, from a single surface placing.

Two combinations of treatments, however, gave promising performances down to the rather limited objective of a depth of 6 in. (15.2 cm.) instead of the 8-9 in. deemed to be requisite. They were (1) a

combination of the Rotospade followed by the Rotary Hoe from two placings — at the surface and 4½ in. (11.4 cm.) deep in the soil, and (2) the Rotary Hoe from two placings 4 in. (10.2 cm.) and 8 in. (20.3 cm.) deep in the soil (compare figs. 4 and 5 with the specification diagram, fig 2).

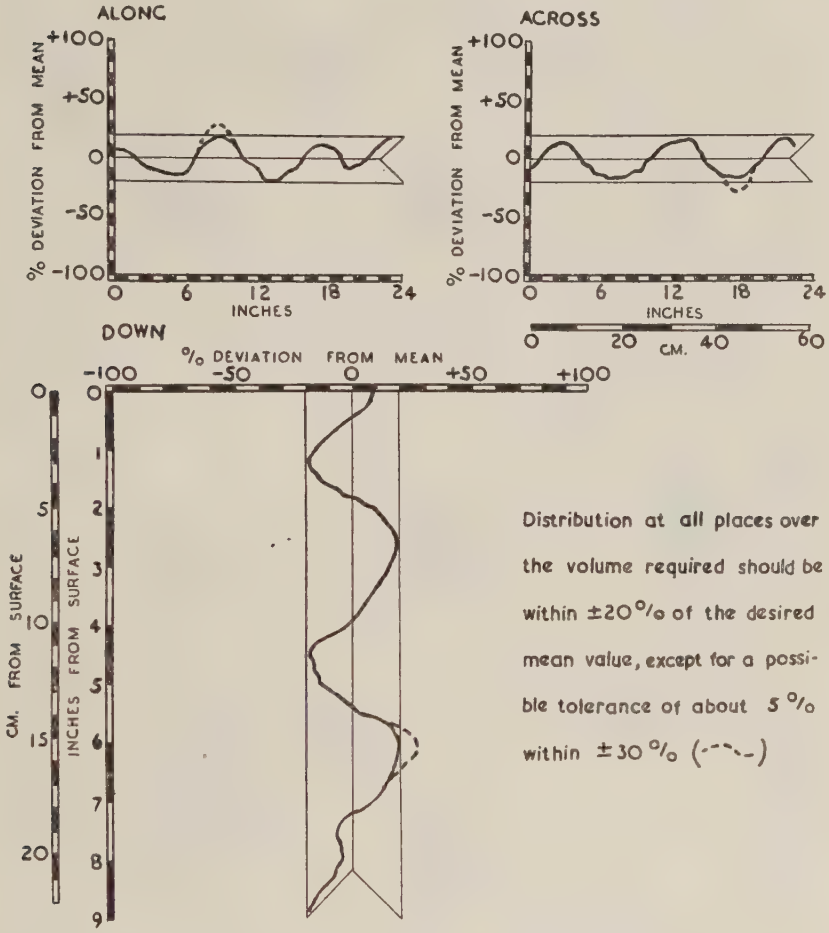

Fig. 2. Specification of adequate mixing for control of *Heterodera rostochiensis*.

Diagrams similar to those of figs. 4 and 5 are useful for a preliminary comparison, but they only compare the distribution at the surface with the *average* distribution in depth. A finer method of assessment is necessary before an operation could be passed as providing an adequate mixing of material in the soil. Deviations from

the mean (i.e. the required rate of application) for *all* readings on each of two vertical transects of soil at right-angles to each other would at least be required. Fig. 6 gives such information for the Rotary Hoe mixing placings 4 in. and 8 in. down in the soil. This shows that it

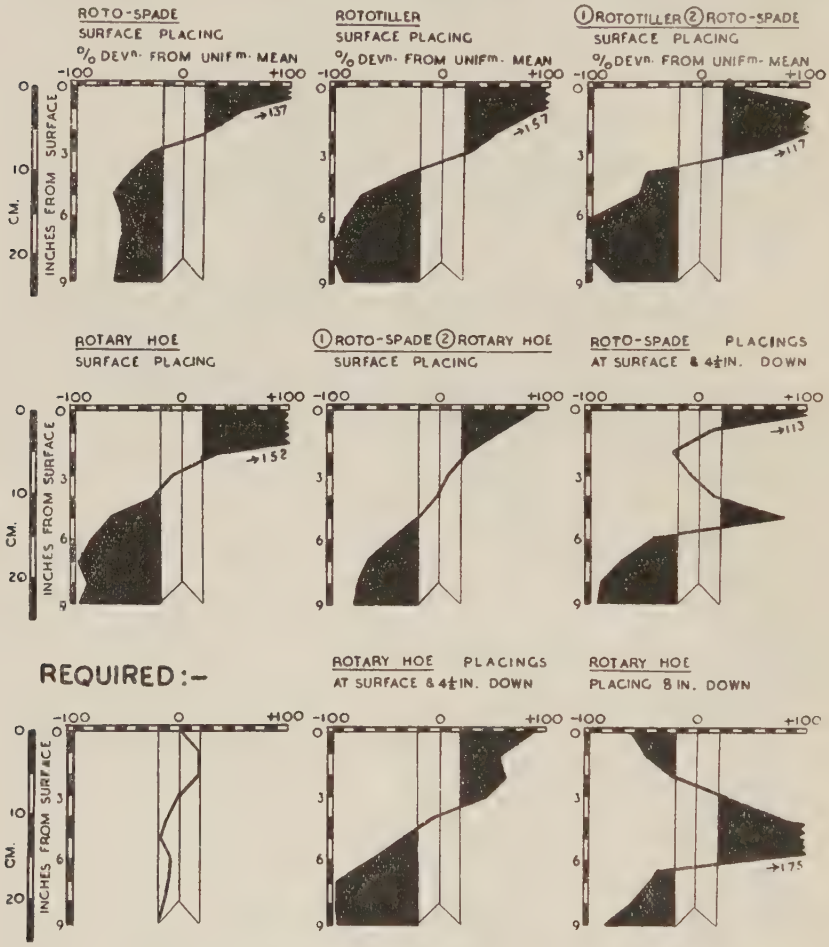

Fig. 3. Unsuitable average distributions in depth.

is possible to get readings within ± 20% of the mean at all depths down to 6 in., though not all readings do indeed lie within such limits.

In working with relatively small plots of treated soil, usually no more than 1 square metre or 1 square yard, certain end and side effects of the mixing device must be taken into account (fig. 7). There are higher readings at the beginning and the end of the traverse of a rotary

cultivator and there are also higher readings at each side (i.e. across the line of travel). The end effects would presumably not be very important in practice when a machine would be taken across the full width of a field. The side effects, however, raise another problem to be over-

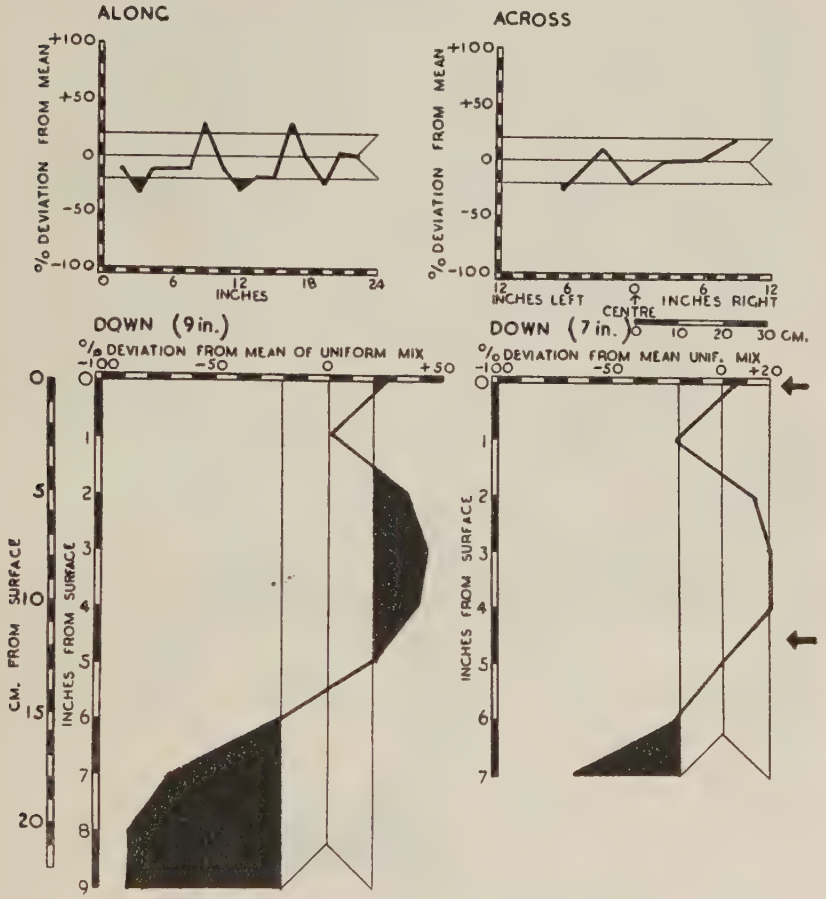

Fig. 4. Average performance diagrams of the Roto-spade followed by the Rotary Hoe, from placings on the surface and 4½ in. (11.4 cm.) down in the soil (shown by arrows, bottom right diagram). The average distribution in depth is acceptable down to a depth of 6 in while the surface distribution is almost acceptable.

come, namely how best to link the treated breadths together so that treatment would be uniform over the whole field. When the end effects were discounted in fig. 6, 43% of the readings lay within ± 20% of the desired mean along the line of travel and 37% across. We may therefore conclude that this particular combination of deep placings

mixed by the Rotary Hoe is roughly about 40% towards the required performance down to a depth of 6 in.

FUTURE WORK

Having obtained a satisfactory average distribution in depth to 6 in.

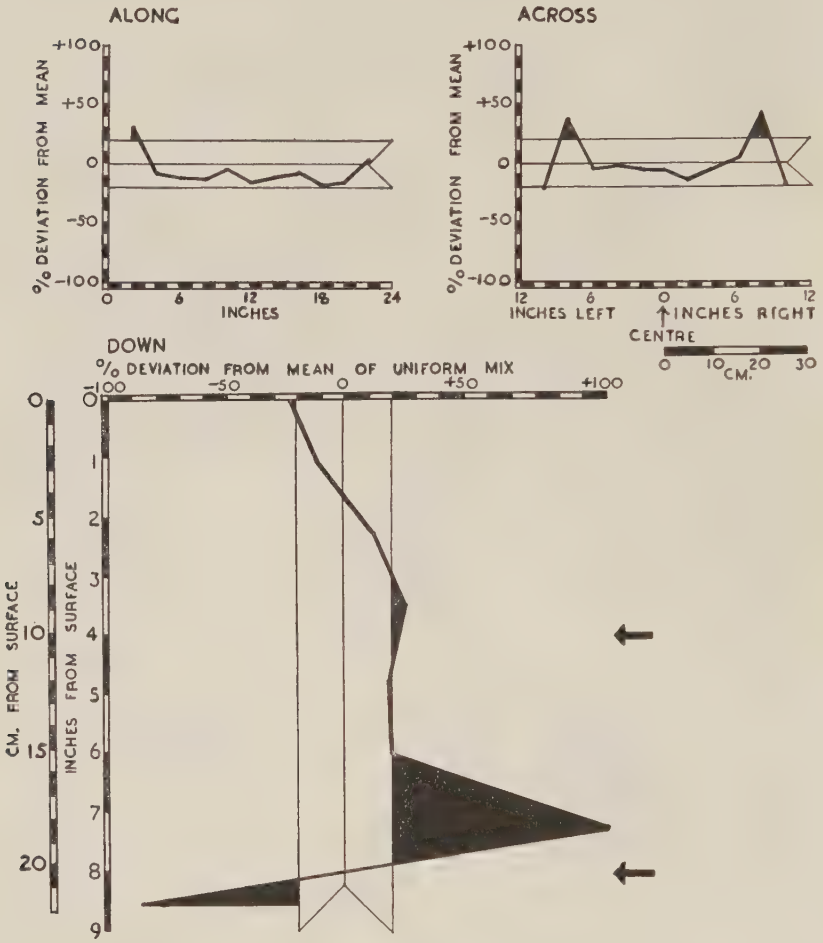

Fig. 5. Average performance diagrams of the Rotary Hoe from placings 4 in. (10.2 cm.) and 8 in. (20.3 cm.) deep in the soil (shown by arrows, bottom diagram). The average distribution in depth is acceptable down to 6 in. and distribution on the surface is almost acceptable.

(15.2 cm.), it should not prove insuperable to extend the economic performance to 8 in., by modifying the depths of placings and of action by the machine. The main obstacle to adequate distribution to full depth

by the Rotary Hoe from 4 to 8 in. placings was an overdose at 7 in. (fig. 5). Placings at two depths have hitherto been of equal strength, but future work should assess the performance of differing amounts. Improvement in the 'individual reading' assessment of fig. 6 might well

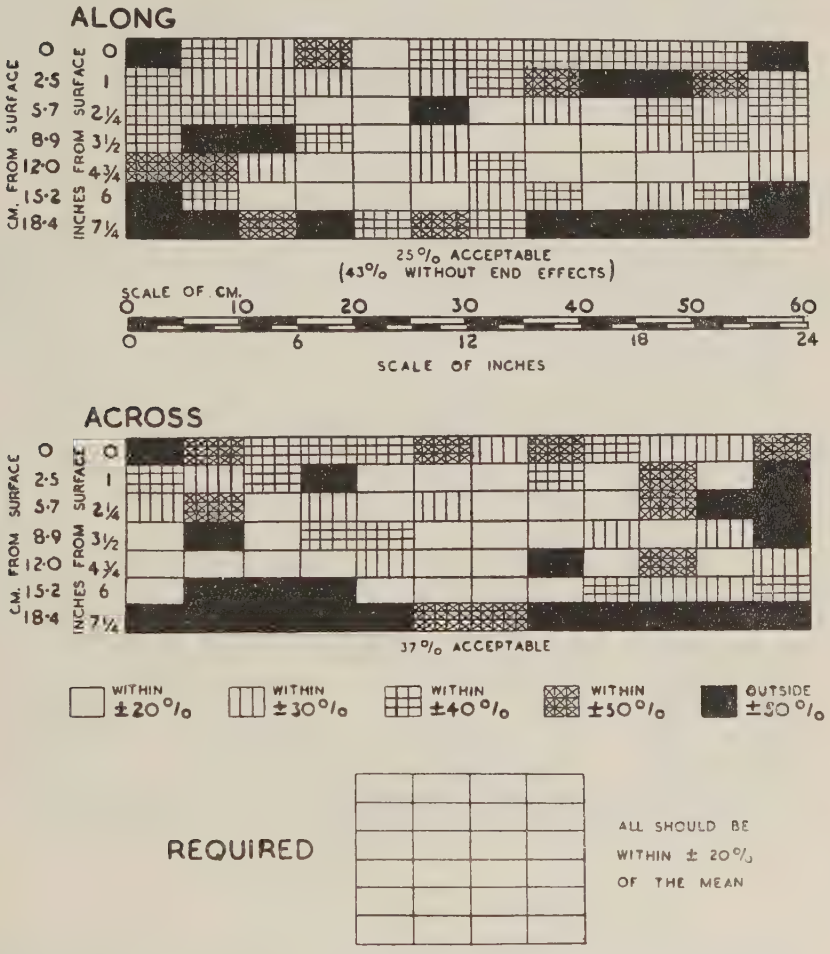

Fig. 6. Individual performance diagrams on two transects along and across the line of travel, for the Rotary Hoe working from placings 4 in. (10.2 cm.) and 8 in. (20.3 cm.) deep in the soil.

be brought about by increasing the rotor speed in relation to the forward speed. The makers of the Rotary Hoe have indeed such a tractor-scale machine in production. One vital development is the mechanization of the initial placing of material in front of the rotor, and to find

whether the material can best be applied as a very low-volume spray or as a dust with very small particle size. The radio-active technique provides a new method for relatively quick determination which is the best of these possibilities.

The ultimate aim can now be visualised as a new farming technique for the potato crop to be grown on eelworm-infested land. A rotary cultivator would prepare the soil to a fine tilth and at the same time incorporate the mercury compound, probably using the fertilizer

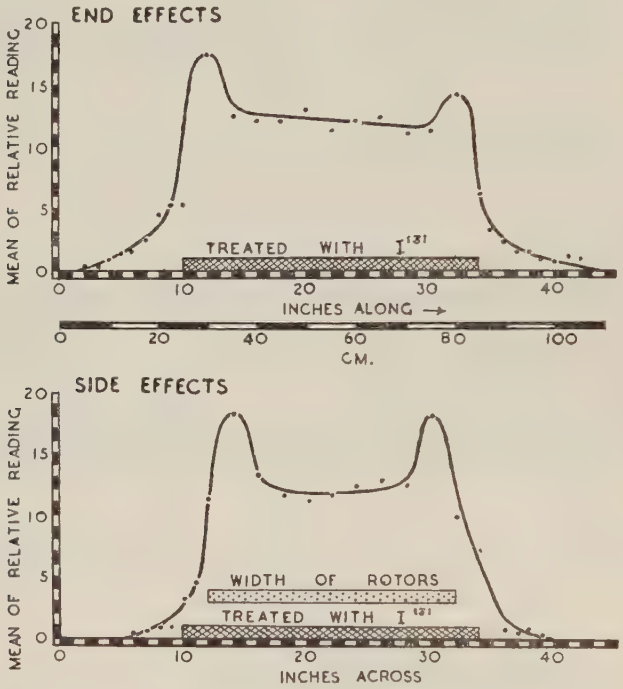

Fig. 7. End and side effects with the Rotary Hoe.

dressing in a special form as the 'inert' carrier of the mercury. Such a combined operation would probably eliminate the expensive item of ploughing and should reduce other cultivation costs. An intimate mixing of fertilizer would, moreover, eliminate from our advisory files cases of potato shoot damage from too great a concentration of fertilizer within the drills. Mercury can, moreover, control more soil diseases than potato root eelworm. The problem requires an integrated approach from plant pathology, engineering, agriculture and chemistry, for the

provision of an inexpensive control of soil-borne disease is an urgent necessity in the modern world.

The writer acknowledges gratefully the help of all members of his staff, the Atomic Energy Research Establishment, Harwell, and Messrs. Thomas Cochran & Co., Ltd., Glasgow, Messrs. Rotary Hoes, Ltd., Horndon, Essex, and Messrs. Wharton & Wilcocks Ltd., Hertford, who provided the Roto-tiller, the Rotary Hoe and the Roto-spade, respectively.

SUMMARY

Mercury compounds used at rates as low as 5.4 lb. mercury equivalent per acre (6.1 Kg. per hectare) have, in pot experiments, given over 80% control of the increase in infestation by *Heterodera rostochiensis* which otherwise takes place when potatoes are grown on untreated, infested ground. Their performance depends on the adequacy with which they can be mixed with the soil, shortly before the tubers are planted. Distribution from solution was best, but required uneconomic amounts of water. The performance of dusts appeared to be related inversely to the particle size of their active agent. Finely ground elemental mercury (grey powder) diluted as a dust, has given control, thus supporting earlier findings by Booer, that mercury compounds are reduced to the metallic element when added to the soil, though still retaining an ability to control parasites.

The degree of mixing necessary for disease control was assessed by using radioactive iodine (I^{131}) in place of the mercury compound. It was found that satisfactory distribution was obtained when the added material varied no more than ± 20% of the desired mean value in any part of the volume treated. In preliminary estimations of average mixing in depth, existing machines fell short of this requirement, when mixing from surface applications. A combination of Roto-spade and rotary cultivator, however, gave promising results from placings at the surface and 4½ in. (11.4 cm.) deep in the soil, though to the rather limited depth of 6 in. (15.2 cm.). The Rotary Hoe alone achieved a similar limited objective from placings 4 in. (10.2 cm.) and 8 in. (20.3 cm). deep in the soil. When subjected to more detailed assessment by individual readings, these mechanical treatments were about 40% acceptable to the limited depth.

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DISCUSSION

H. GOFFART: asked if mercuric chloride would be stored by plants.

J. GRAINGER: This is unlikely, because mercury compounds are reduced in the soil to elemental Hg which would not be taken up by the plant.

D. ROBERTSON: asked if Dr. Grainger could give some idea of costs of:

1) Material — i.e. mercuric chloride — per acre.

2) Application of material per acre.

J. GRAINGER: The cost of 1) would probably be £ 3 to 4 per acre for material, depending on the price of mercury. It compares with £ 15 for 200 lb, of D.D.

The cost of 2) cannot yet be estimated with certainty, but it is the aim of future work to keep it low, as suggested in the last paragraph.

J. D. BIJLOO: Does Dr. Grainger know anything about the further action of mercuric chloride or mercury grey powder on other "wanted" microorganisms in the soil? My suggestion is that we should work with nematicides that decompose or evaporate as soon as possible from the soil, in order to influence the ecological situation in the soil as little as possible.

J. GRAINGER: BOOER (1951) suggested that mercury vapour diffused from the metallic element, to delay the development of the parasite more than that of the host, thus disturbing the relationships necessary for disease to occur. Mercury vapour is stated to have no direct destructive action on fungi or eelworms, so that until a host plant is also involved, it would presumably not have a bad effect on the "wanted" organisms.

H. R. WALLACE: Experiments with beet eelworm indicate that low concentrations of mercuric chloride may stimulate hatching.

J. GRAINGER: I agree that this may be so, and, moreover, 0.01% mercuric chloride solution also keeps larvae of *Ditylenchus dipsaci* alive for a longer time than does water, hence the need for a hypothesis to explain how mercury compounds can be reduced to the element in soil and still retain a capacity to control disease.

C. ELLENBY: It seems to me that the toxicity of mercury vapour has been underestimated. It is remarkably toxic to human beings and certainly to insects; some work on the respiration of insects may have been influenced by minute traces of mercury used for calibrating the apparatus.

J. GRAINGER: The amounts of mercury are extremely minute in relation to the volume of the soil (1 of Hg to 414,815 of soil), thus reducing any direct toxic effect. There is already circumstantial evidence that larvae are not killed after soil treatment with mercury compounds, but many details of their mode of action must await further work.

(*Nematologica*, Vol. I, 1956, no. 1)

AIMS AND METHODS IN POPULATION RESEARCHES ON SOIL-INHABITING NEMATODES

BY

G. MINDERMAN

(Institute for biological field research, Arnhem)

As a member of the staff of the Institute for biological field research I shall begin by explaining the work that is covered by the mandate of this Institute. It is our task to carry out research in applied biology in the broadest sense of the word in the field. By the term "applied" we mean that our research is aimed at obtaining a better understanding and mastering of nature to the ultimate advantage of mankind. Much of the research carried out at our institute is fundamental in its origin, but is all the same directed towards a practical end. The workprogram mentions for example research on the influence of hedges and windscreens on the yield of agricultural crops; on mammals and birds; on the decomposition of organic matter in the soil, and on the problem of regulation and stabilisation processes in natural communities. In this latter problem we ask ourselves what the factors or processes are which protect such natural biotopes against those pests that so often attack our cultivated fields. The nematological research comes under this heading. The aim is to gather data on the biotical and abiotical factors governing the soilfauna. We may then be able to understand the faunistic composition, the changes, and the regulation of population density of the nematodes in the soil. We must trace the existing relation between the nematodes themselves and their environment. To achieve this aim we often need a quantitative analysis of a biotic community.

When starting our research we assumed that a biotic community in a natural biotope is better buffered than that in a cultivated field. The term "buffered" is used in the sense that organisms affect each other in such a way that it is impossible for any species to increase to plague proportions; on the other hand the species do not prevent each other from living. It is our opinion that it is best to carry out such research in a biotope as natural as possible; we may then become acquainted with the factors and processes buffering the community. For practical reasons we prefer a biotope which remains constant

throughout the year and changes but little from one year to another. The best approximation of our ideal seems to be an old-established heath, since here, there are large areas covered exclusively by Calluna without any other plants growing under it. Moreover, its leaves are shed throughout the year and growth stope only when the heath is frozen.

It is open to questioning in how far a heath represents natural conditions but in places were it has been established for about 2000 years, it is sufficiently stable for our purposes.

A second choice was an oak forest where the yearly leafshed is decomposed within one year.

We appreciate that conditions in these two cases differ from those occurring in cultivated fields which are of course subject to disturbance by tilling, fertilization or grazing. It is however hoped that from a study of the processes in more natural biotopes it is possible to gain sufficient knowledge of the principles involved so as to be able to study further the problem in disturbed soil.

To obtain reliable quantitative data we had to develop new techniques that would overcome the difficulties encountered in analysing samples containing a high percentage of humus.

In the beginning we examined a number of techniques already described in literature, but none of them were able to satisfy us. It was most desirable to be able to extract all nematodes, including the smallest from any given sample. The method proved to be the direct counting technique described by Stöckli.

This process however is both very tiring and time consuming. We clung to its principle but subjected the samples to staining before counting. This way the nematodes contrast strongly with the soil particles and are easily extracted by hand. Using this technique many samples were examined, as well as by other methods which were tested for their reliability. In routine work the time factor is of the highest importance, and for this reason we were looking for a reliable but much quicker technique. We did find such a technique but had to accept an efficiency of 95 %-99 % in ascertaining the number of nematodes present in the tested sample.

This technique proved to be 8-10 times as fast. It is based on suspending the particles of a soil sample in a salt solution of high specific density, followed by a process of centrifuging.

This technique worked well on small samples of any kind of soil tested by us. Besides the examination of soil, we also examined the litter.

We succeeded in obtaining fairly reliable results by intermittent shaking of a sample immersed in water. After shaking it proved possible to check the number of nematodes still present in the leaves, by bleaching the leaves in a solution of hydrogenperoxyde followed by staining, with the result that the nematodes, fungi and bacteria could be studied with high power lenses under the microscope. In all these methods the organisms were separated from their substratum in order to determine their presence. It will be appreciated however, that we place great value on the study of the organisms in situ in their substratum. For by so doing we should get an idea of the environment needed for the different species; e.g. the size of the capillaries they live in; the different organisms living side by side and the over-all distribution of the different species. KUBIËNA was the first who tried to solve part of these problems by making sections of the soil after embedding it in resins or plastics. This process has the drawback of dehydrating animal tissue to such an extent as to make it impossible to identify the organisms.

HAARLØV and WEIS-FOGH also tackled the problem with some success but their sections were too thick (500 micra or more) for a detailed examination.

Using gelatin as the imbedding substance and dissolving the sand grains by means of fluoric acid it is possible to cut sections of soil in its natural state of 10 micra thickness. It is possible in these sections to distinguish the different organisms well by using appropriate staining techniques. Although this technique is as yet not faultless, first results are promising.

From this paper it may be seen that as a consequence of the research design our attention will not be directed to any special group, not even to the economically important group of the *Tylenchidae,* but to soil nematodes in general and to other organisms connected with them. In short our aim is to obtain an insight into what goes on in the soil, in and around the group of the nematodes.

We hope in this way to contribute something to the broadening of agriculture scientific knowledge.

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DISCUSSION

J. H. SCHUURMANS STEKHOVEN: 1) Can quantitative differences be distinguished between the types of soil mentioned?

2) Are any experiments done with fumigants?

G. MINDERMAN: 1) Large differences exist between soil from heath and soil from woods, especially quantitative and faunistic.

2) Fumigants are not and will not be used.

D. ROBERTSON: asked for more details of the technique for cutting microtome sections of soil.

G. MINDERMAN: The procedure was mentioned shortly, a detailed description will be published.

T. D. WILLIAMS: Requested more details of the technique for separation by centrifugation — particularly the rate and duration of centrifugation.

G. MINDERMAN: As a rule 1 minute at 1500 revs/min.

N. S. PITCHER: Are you yet able to suggest the nature of any of the factors having an important effect on the balance of soil nematode populations?

G. MINDERMAN: No, as yet it is impossible to give any indication.

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ELECTRONIC FLASH IN PHOTOMICROGRAPHY

BY

C. C. DONCASTER

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In the British periodical "Discovery" of November 1953, there appeared an article by D. P. WILSON (1953) entitled "Electronic Flash Photomicrography". Here the author described a method of using for photomicrographic work the ordinary portable electronic flash units of about 100 joule output which are employed by press photographers and others. Obviously to use this sort of general purpose equipment would be a much more economical proposition than to purchase special high speed flash apparatus for photomicrography such as that designed by R. H. J. BROWN (1952). BROWN's equipment employs a special electronic flash tube in which a concentrated flash occurs at a position where a constant arc gives light for observation and focussing. WILSON, however, used a Mullard LSD. 3. flash tube; the type with a large helix about 4 cm. long in which the flash occurs. It has a flash duration of .1 to .2 of a millisecond and dissipates only 100 joules. He placed the tube horizontally before the microscope mirror and over it leant a piece of ground glass, the polished side uppermost. Above the ground glass he placed a 60 watt opal lamp, the light from which was reflected by the glass to the mirror and thence through the optical system of the microscope. Above the microscope he used an ordinary photomicrographic camera with shutter and viewing eyepiece. Most of his work was done on living marine plankton organisms and with this equipment he could view his specimens up to the moment of taking the photograph. Exposures were made by pressing an electrical foot switch which first put out the viewing light, then released the camera shutter and simultaneously fired the flash.

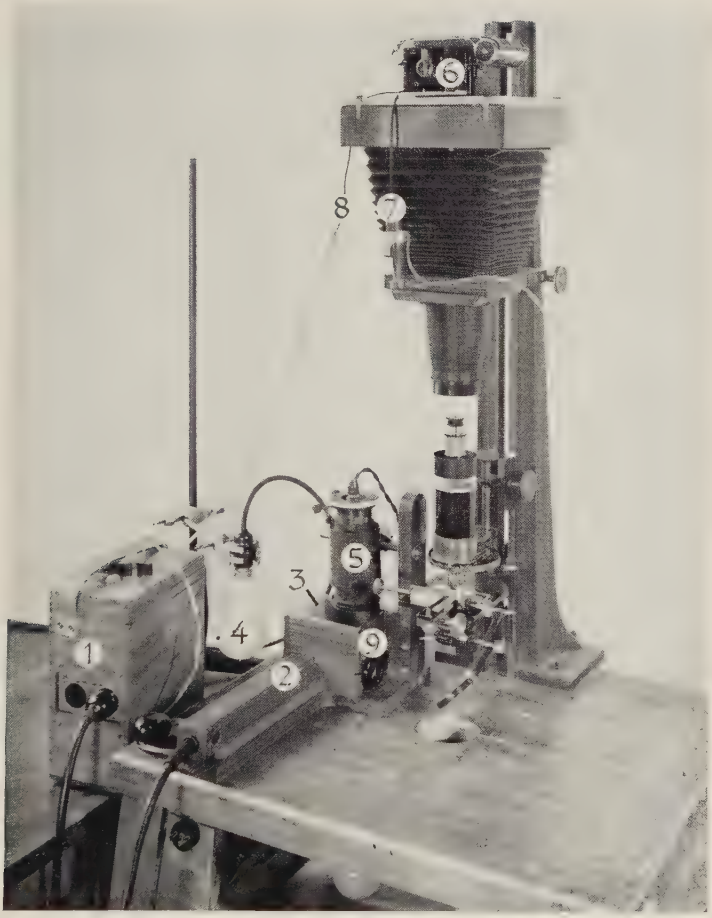
In the spring of 1955 after collecting some nematodes from the root system of a *Brassica* plant in a Baermann funnel, I found the culture seething with the large ciliate protozoon, *Stylonichia pustulata*. The funnel had by this time been standing for a few days and in its stem there were many moribund nematodes, probably suffering from a shortage of oxygen. The protozoa, however, were still active and many of them were attempting to feed on the immobile nematodes.

Had electronic flash equipment then been immediately available I should have been able to record this apparently unusual occurrence. However, within less than an hour the nematodes, which I had drawn off into a shallow dish had begun to revive and the protozoa were no longer attempting to feed on them. While this was by no means the only incident of interest which we could not have photographically recorded with the ordinary type of apparatus, it did help to show us the value of having the sort of equipment used by WILSON immediately available. Within a few days our carpenter at Rothamsted had made an adaptor to take an ordinary 100 joule flash unit on the bench of our photomicrographic apparatus (Pl. VIII).

The reflector supplied with the equipment is removed from the handle flash-tube holder and the tube inserted into a box. This holds a piece of ground glass at 45 degrees over the tube and a removable plate with a field iris in front. In the back of the box in line with the centres of the flash tube and field iris, is drilled a hole about 2 cm. in diameter. Behind this is placed a lamp which is used only for centering and aligning the microscope mirror and condenser. For this operation the flash tube is temporarily removed from its housing to uncover the centering light. Having adjusted the mirror to the correct height to come into line with the field iris and centering light and having centered the condenser as for ordinary microscopical work, the position of the auxiliary viewing lamp, which is placed vertically over the ground glass, is then adjusted. To do this the ground glass is slid down into position in front of the flash tube and the viewing lamp moved along its supporting rod until it completely illuminates the field. The reflection of the viewing lamp in the ground glass and the light from the flash tube behind it are now exactly in line with one another and either of them will illuminate the subject.

The substage condenser is set so that a plane slightly in front of the field iris is brought into focus with the specimen. This is simply to eliminate the pattern of the ground glass from the picture. The field iris can still effectively reduce the area of illumination to fill only the field of vision and to cut down glare.

Having selected and fitted the appropriate objective and ocular, the camera is then lowered into the light trap at the top of the microscope tube and its length adjusted to give more or less the correct total magnification to do justice to the objective in use. The camera I use is an Agiflex, an English single lens reflex camera, taking 6 x 6 cm. pictures. The whole lens panel is easily removable and if this is done



The photomicrographic apparatus with electronic flash equipment.

1. Electronic flashlight unit (power pack); 2. Flash-tube holder; 3. Ground glass diffusing screen and "mirror"; 4. Centering lamp; 5. Viewing lamp; 6. Viewing and focussing screen of reflex camera; 7. Shutter release and switch for putting out viewing light; 8. Shutter-flash synchroniser lead; 9. Field iris.

Electronic flash photomicrograph of *Dorylaimus* species recovered from the vicinity of grass roots. Photographed with 16 mm. objective and 4½ × ocular.

cut-off on the picture is reduced and the best use made of the negative area.

Reflex cameras of this type have a "focal plane" shutter placed immediately in front of the film and the image of the subject is reflected onto a viewing and focussing screen by a hinged mirror resting at an angle of forty-five degrees to the image-forming beam. But when the shutter release cable is pressed, the mirror is moved out of the beam, the shutter exposes the film and simultaneously triggers off the flash. It is possible, therefore, to watch and focus on the living specimen right up to the moment of taking a photograph. However, it is important to extinguish the auxiliary viewing light just before an exposure is made, in order to avoid a double image appearing on the film due to movement of the specimen and to the two sources of illumination: the flash and the viewing light. A switch has therefore been provided on the shutter operating cable and as soon as the cable is pressed, the viewing light goes out and immediately afterwards the shutter is released, triggering off the flash and casting an image of the specimen onto the film for less than .2 of a millisecond. This very brief exposure will show no blurring of the image due to movement.

The choice of microscope lenses will vary according to the size of organisms to be photographed. For large nematodes a 24 mm. or even 56 mm. objective is very useful, coupled with a low power projection eyepiece of about 5 cm. focal length. With these lenses and medium fast film of about 27° Scheiner it is necessary to interpose a filter in order to reduce the light intensity to 20 to 30 per cent. of its normal strength. With 16 and 9 mm. objectives no filter is necessary and with a 4 mm. objective it is necessary to use a high sensitivity film in order to obtain adequate exposure.

Although the camera length should theoretically be adjusted to a definite amount for a given lens system it is not always practicable to do this and the magnification may have to be checked at the beginning or end of a series of photographs. I now make a practice of concluding a series or a film, whichever ends first, with a photograph of a 1 mm. stage micrometer, using of course, the lens system and camera length employed for the series. If lantern slides are to be made and it is important to show the degree of magnification, the picture of the stage micrometer can be printed on another slide which is placed face to face with the subject and used as a cover glass. If a print is to be made the scale can either be over-printed beside the picture of

the specimen, or simply marked off in pencil on the back or margin of the finished print.

Pl. IX shows the head end of a large *Dorylaimus* species collected from the vicinity of grass roots. It was photographed with a 16 mm. objective and a Watson 4½ times projection ocular. It shows that a fair amount of detail can be obtained in photographs taken by this technique, although it is more difficult to get critical overall focus due to movement of the specimen.

There is undoubtedly a case for obtaining pictures of this sort, of any unidentified specimens which are to be killed, fixed and mounted for a critical examination, because distortion or major artifacts resulting from the treatment may be discovered by reference to the photograph. Moreover accurate measurements may be taken of living nematodes, spears, spicules etc. if it is believed that any appreciable distortion may result from killing and fixing.

Specimens are mounted on a flat (not a cavity) slide in a drop of water and I have found it quite satisfactory to drop a cover glass over them without using props to take the weight. It is very important to restrict movements of the specimens to one plane, as far as possible, so that definition remains good over the whole body. With very active organisms this is easier said than done, but by carefully drawing off some of the water from the mount with filter paper, nematodes can usually be slowed down without doing them any harm. It is then not so imperative to keep both hands on the mechanical stage in order to keep the organisms in the field. Focussing can be checked with one hand, and by using the focussing magnifier on the camera. The other hand is ready to release the camera shutter the moment the whole specimen appears in focus.

One has to be prepared for a high percentage of out-of-focus shots and I usually find it necessary to use up a whole film of 12 exposures on one nematode to be reasonably sure of a single good result.

Cleanliness in the preparation of a mount makes all the difference to the clarity and aesthetic quality of the photomicrograph: it is always advisable to rinse the specimens well in a drop of clean water before transferring them to the mounting drop. Slides and coverslips should be thoroughly cleaned and dusted before use.

Dark ground illumination may give the most successful results with delicate, transparent organisms, but I doubt if it has any advantage in the photography of nematodes. If this type of illuminator is to be used, the exposure has to be increased to obtain a sufficiently strong

negative and no filter is used even with low power objectives. With the higher power objectives a special high speed developer such as Johnson's "Capitol" may be necessary.

Another possible use for the electronic flash technique is the measurement of nematodes from fair sized enlargements of the organisms themselves and of the stage micrometer at the same magnification. Fuse wire bent to the shape of the eelworm or perhaps better still a map measurer may be used to obtain accurate measurements of the pictures of the living worms. With this method there is no possibility of distorting the nematodes which may occur if they are killed by heat treatment, for example.

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SOME OBSERVATIONS ON HETERODERA MAJOR

BY

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There appears to have been an increase in the incidence of damage to cereal crops by *Heterodera major* in Great Britain since the war. This increase may be due in part to a greater awareness that this eelworm can cause crop failure, but it seems certain that the cereal root eelworm, which has been found to be widespread in England and Wales, is potentially a dangerous pest.

An increased study of *Heterodera major* began at Rothamsted with a search for a Root Diffusate hatching agent, the testing of varieties of oats and barley for resistance to the eelworm, and an investigation into eelworm population build-up under various cereal hosts. The techniques applied to the extraction of cysts from soil, their hatching, counting and testing in pots were based on those found successful when used with the potato-root eelworm, *H. rostochiensis* but post season cyst counts of *H. major* cysts in pot tests showed that the eelworm had scarcely multiplied on its known-hosts.

In the following season cysts of *H. major* were obtained from moist soil by a sieving technique. The cysts were not allowed to dry, and they were found to hatch fairly readily in tap water, at room temperature. WINSLOW (1955) has shown that dried cysts of *Heterodera major* do not hatch well; in an experiment carried out at Rothamsted, WINSLOW's results were confirmed and it appears that this eelworm is quite sensitive to drying. Twenty replicated batches of one hundred cysts were subjected to a drying treatment in a 25°C. incubator after the cyst batches had been found to be hatching in water. Excess water was drawn off the cysts with a pipette before the drying treatment, which consisted of exposure to the drying atmosphere within the incubator for periods of 1, 2, 4, 8 and 16 hrs. The cysts were re-immersed in water after their respective treatments, and kept at the same temperature. It was found that the hatch was not reduced by the 1 and 2 hours drying treatments, but after 4 hours drying there was a reduction in the number of hatched larvae, and after 8 and 16 hours drying, no larvae emerged from the cysts.

TABLE I

Heterodera major Larval hatch after drying of cysts

Treatment (Drying time in hours)	Larvae hatched/100 cysts in eight days			
Replicate	A	B	C	D
1	580	1390	600	720
2	460	840	1550	600
4	60	760	450	10
8	0	0	0	0
16	0	0	0	0

All the cysts were kept in water which was changed each week until November, when the remaining egg content of the cysts was investigated. From May, when the experiment was carried out, to November, seven larvae emerged from one batch only of the cysts which had been dried for 8 and 16 hours. The hatch in batches of cysts dried for 1, 2 and 4 hours ceased after four weeks, at the end of June. Attempts to re-stimulate hatching by keeping all the cyst batches at a temperature of 2°C. for two weeks during August and replacing them in temperature of 25°C. failed to produce further larval emergence. While this experiment was concerned with the drying of the cysts, it is suggested that it is the amount of drying received by the larva within the egg that determines whether or not its hatchability is effected and under certain conditions not all the eggs within the cyst may be affected by drying.

Franklin (1940) has shown that *Heterodera punctata* cysts will not hatch if they are dried; it is interesting that *H. major* and *H. punctata* are both parasites of grasses and cereals only.

Heterodera cysts are usually extracted from soil for purposes of population estimation, or to obtain living material for experiments. Because of the effect of drying on the cysts, it may be necessary to use different techniques for cyst extraction when *H. major* cysts are required. The soil washing technique using the Fenwick can (Goodey 1951) may not be satisfactory when an estimate of the population of *H. major* is required, and a modification of the usual process is necessary. This is because the new and full cysts of *H. major*, especially those covered with the subcrystalline layer, do not float readily. They may float when dry, being held afloat by the surface tension of the water, but once they are thoroughly wetted they will sink. Passage through the Fenwick can may wet these cysts enough to make them sink, and if the cysts and debris from the can are

refloated at once in a flask to allow silt to settle, many cysts may be lost: losses of up to 70% of cysts have been observed at this stage in soil washed in September. The modification of the technique is to thoroughly dry the float from the Fenwick can prior to its refloating, which must be done by scattering the float on to the surface of water in a flask. The cysts and other floating and suspended material are decanted on to a fine sieve or bolting cloth after the silt has settled to the bottom of the flask. To minimise or prevent the loss of *H. major* cysts in the Fenwick can it is important that the soil sample is perfectly dry, and is washed through the can rapidly so that cysts do not become completely wetted before they enter it. Cysts of *H. major* picked or rubbed off dry oat roots in September have often been found to be very moist within, the eggs being surrounded by mucus. Some of these cysts stored in dry specimen tubes in the laboratory remained moist until they were opened three weeks later. Wet mucus is not found in older dried cysts of *H. major,* and its presence in new cysts may be a factor which affects their floating. It is advisable to take soil samples in the winter months when the mucus seems reduced in quantity perhaps as a result of bacterial or fungal action. Cysts of *H. major* are known to be parasitized by several species of fungi. (GOFFART 1932).

To provide a stock of hatchable *Heterodera major* cysts it is necessary to wash moist soil in a Fenwick apparatus, and to recover cysts from the float by elutriation. Soil collection and part-drying is best done in the winter (January is a convenient month for this in England). The soil sample is allowed to become almost dry, and is mixed frequently throughout its exposure to drying, to prevent cysts on the surface of the soil from becoming so dry that they will not hatch. When the soil crumbles easily in the hand, it may be washed. If soil is collected and part-dried in March and April, the drying process, which usually involves making the soil warmer, may stimulate hatching, and some of the cyst content will be lost. The soil is washed through a large Fenwick apparatus as rapidly as possible and is added in such quantity that it does not block the top sieve and become thoroughly wetted before passing into the can. The float is collected on a 60 mesh/in. sieve, and never allowed to dry. It is washed through sieves of 16, 25, 30, 40 and 50 mesh/in. and the material in the 16 and 25 mesh sieves is discarded. The 25 mesh sieve needs careful washing because it may retain a few extra large cysts. The cysts and debris in the 30, 40 and 50 mesh/in. sieves are transferred to a covered

shallow dish and kept at a temperature of about 2° C. At this temperature the cysts hatch only slowly. The cysts in the float are removed

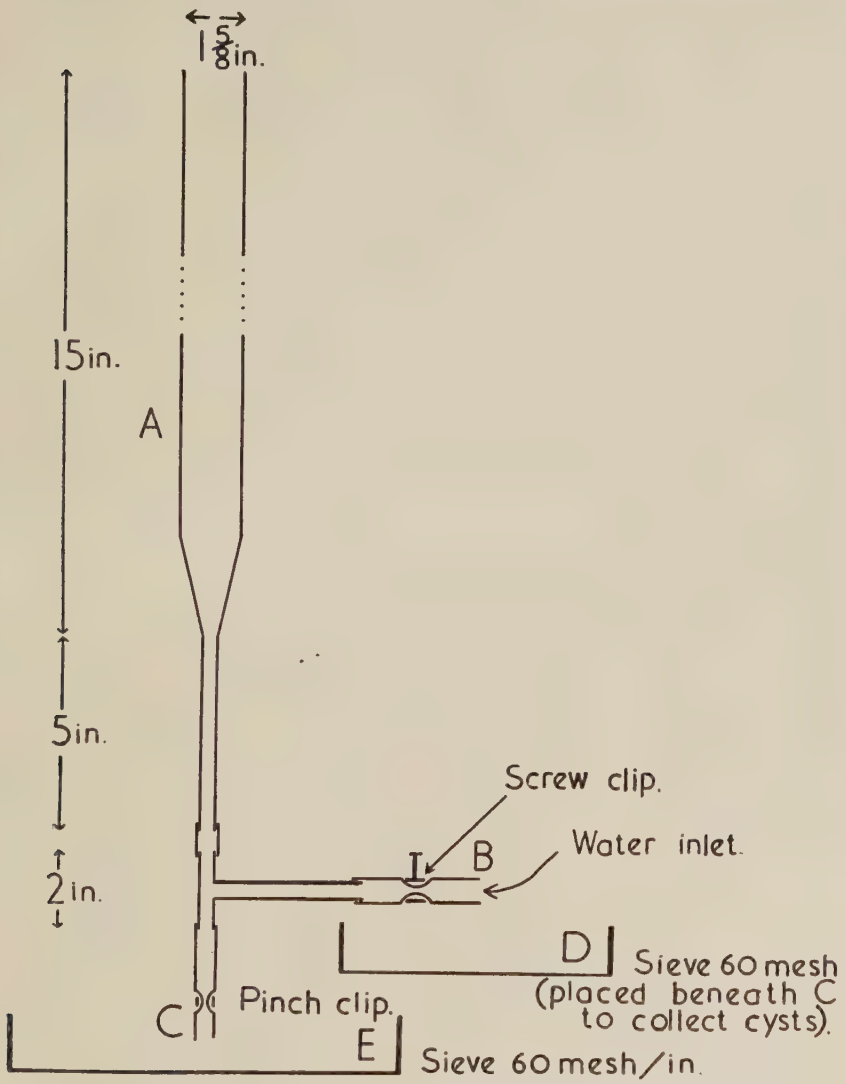

Fig. 1. *Heterodera major.* Elutriator for extraction of wet cysts.

from cold store for elutriation in the apparatus shown (fig. 1). In operation the tap C is closed and water is allowed to run through B into the glass tube A. A very gentle and finely controlled flow of water is necessary, and small quantities of the cysts and debris are

put into the tube A. Where there is a lot of debris it may be necessary to stir the contents of the tube A to prevent cysts from being carried out of the apparatus within clumps of suspended material. A gentle flow of water carries off such debris as rootlets, and some cysts, but these will have a low egg content. The flow of water is increased by very small amounts as the cysts become separated from the debris.

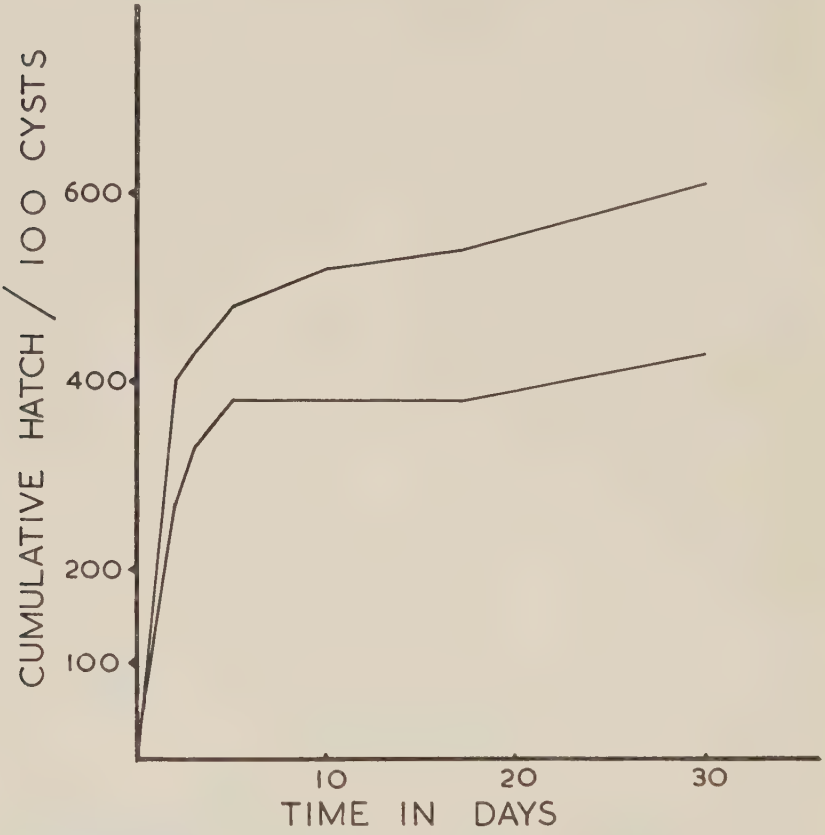

Fig. 2. *Heterodera major*. Larval hatch at 25° C. (two replications).

until the water is almost clear of suspended material. The cysts are found at the bottom of the tube, suspended there by the gentle upward flow of water. To remove the cysts, tap C is opened and the cysts run out into a 60 mesh sieve D placed temporarily beneath the outflow tap at C. The sieve E collects all the overflowed material. Cysts collected in the sieve D are transferred to a covered glass vessel containing wet filter paper and stored at 2° C. Cysts obtained by this elutriation

technique, especially those of the largest grade, are usually full of eggs and should be good experimental material.

It has been mentioned that the cysts should be stored at a low temperature. A series of hatching tests, conducted at different tem-

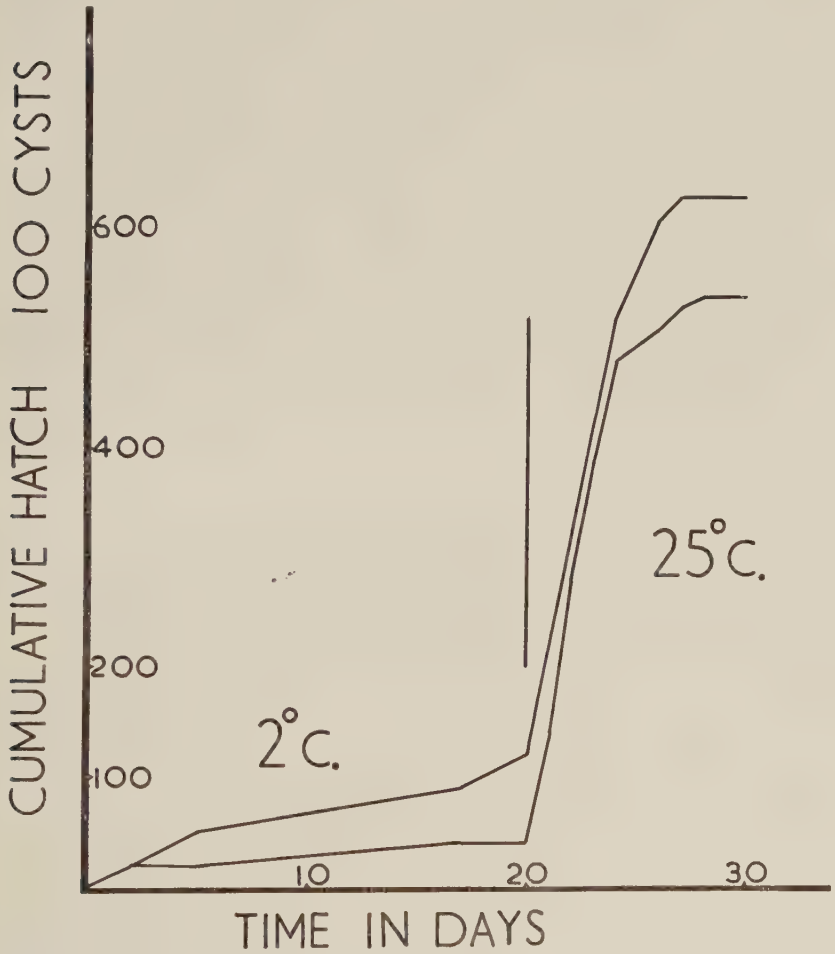

Fig. 3. *Heterodera major*. Larval hatch from cysts at two temperatures (two replications).

peratures, showed that the larval emergence from *H. major* cysts varied with temperature. The temperatures used were 25° C. and 2°C. followed by 25°C. At 25°C a graph (fig. 2) of the cumulative hatch plotted against time showed a high rate of hatch maintained for 6-8 days only, 2390 larvae emerging in 30 days from 490 cysts with an average

content of 51 eggs, an average hatch of 9%. Such a graph (fig. 3) plotted for the hatch from cysts kept at 2° C. showed that the rate of hatch was low, only 470 larvae emerged from 500 cysts in 20 days. These cysts had an average content of 49 eggs. Cysts transferred from a temperature of 2° C. to a temperature of 25° C. showed a rise in the rate of hatching which again fell off after about 6 days, the average hatch in this case was 13 %.

It is interesting to compare the number of larvae hatched in these experiments with the hatch recorded in infested soil kept fallow in plant pots. Here, a population of 246 la/g./ fell to 83 la/g./ between 20 March and 7 July, i.e. a fall of 75% and 12 April of the following year, the population had fallen to 63 la/g./

WINSLOW showed that the hatch of *H. major* was greater at a temperature of about 20°C., than at 25°C. A temperature of 25°C., which has been observed to be the best temperature for conducting hatching tests on the potato root eelworm, may be too high to maintain the hatch of larvae from cysts of *Heterodera major,* though raising the temperature of moist cysts to this figure certainly stimulates hatching.

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DISCUSSION

H. R. WALLACE: There appears to be a series in species of *Heterodera* with regard to a) water-hatch and b) reaction to drying. *H. rostochiensis* has a low water-hatch, *H. schachtii* a higher waterhatch and *H. major* even higher. In addition hatch in *H. rostochiensis* is least affected by drying, *H. schachtii* is more affected and *H. major* affected most of all. A correlation between these two phenomena may be significant.

J. J. HESLING: This is a most interesting observation; it seems also from the observation of FRANKLIN (1940 — J. Helm. 18 (2/3) pp. 63-84) that *H. punctata* may be more affected by drying than *H. major.*

B. HOMEYER: Die Apparatur arbeitet nach dem Gegenstromprinzip. Bei welchem Wasserdruck erfolgt die beste Trennung der Zysten von den Humusbestandteilen?

J. J. HESLING: The waterpressure requires a very fine and critical adjustment which varies (under the control of the operator) as the cysts and debris separate. A definite water-pressure cannot therefore be given.

H. GOFFART: Wir haben beobachtet, dass die Larven von *H. major* aus einigen Zysten sehr stark, aus anderen nur sehr schwach schlüpfen. Hat Mr. Hesling Ähnliches beobachtet?

J. J. Hesling: No, I have not seen this, probably because my investigations have been on batches of cysts, and not on single cysts.

P. Bovien: The larvae cannot escape through the vulva proper but they will easily be able to make their exit through the thin-walled areas found dorsally and ventrally to the vulva as soon as the membranes covering these areas are broken. The age of the cysts is therefore of great importance in this connection. The larvae can of course also escape when the neck is broken.

J. J. Hesling: At Rothamsted larvae of *H. major* have been observed emerging simultaneously from the neck and vulval end of cysts. I agree with Dr. Bovien that the age of the cysts, with regard to the opening of the vulval fenestra, is important in regard to larval emergence.

I would add that the maturation of the larvae within the eggs is also an important factor where hatchability is concerned.

W. J. van der Linde: Does Mr. Hesling know whether the dried cysts are dead or could they be brought to hatch by some root extract?

J. J. Hesling: It is very probable that some of the dried eggs within the cyst are killed and a fall in egg-content of cysts subjected to various drying treatments has been observed, when there has been no or very little hatch afterwards. The eggs may disappear as a result of fungal or bacterial action.

It is difficult to know whether a given drying treatment on a cyst has affected all the eggs by the same amount.

A root diffusate hatching agent is unknown in the case of *H. major*.

(*Nematologica*, Vol. I, 1956, no. 1)

NATIONAL SURVEY WORK FOR CEREAL ROOT EELWORM

(*Heterodera major* (O. SCHMIDT) FRANKLIN)

BY

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Cereal root eelworm (*Heterodera major*, syn. *H. avenae*) was first discovered in Britain by THEOBALD, who recorded it on oats in Lincolnshire in 1908 and on wheat in Kent in 1909. In 1933 and 1934 EDWARDS reported the first serious cases of damage to oats in the West Midland counties of England (FRANKLIN 1951). During the following years it became clear that the eelworm was well established in this district and scattered cases occurred elsewhere. The eelworm was soon recognised as a potentially serious pest. Since the end of the Second World War, damage has increased considerably and by 1952 it was clear that we were dealing with a problem of national importance. This situation may be due to some extent to an increased awareness of the problem, but it is no doubt mainly the result of increased cereal growing during the war, when many acres of old grassland were ploughed.

Reports from entomologists of the National Agricultural Advisory Service (ANON. 1953) showed that the West Midland district was the worst affected. Damage by the eelworm had also become commoner in some of the northern counties, where it had been known for about fifteen years. In the south there had been only a few records before the war, but a rapid increase had now occurred, especially on the chalk hills of Hampshire and Wiltshire — this district in particular is one where large areas of former sheep pasture were changed to arable cultivation during the war. Wales is not as yet very much affected, except for a district which is geographically adjacent to the badly infested West Midland counties. The eelworm is known in Scotland, mainly in the east, but it has not yet been found in Northern Ireland.

PILOT SURVEY, 1953

In 1952, it was decided to undertake a national survey to gain more information on the distribution and status of the pest. It was found,

however, that we needed more information on field and laboratory techniques for the detection and assessment of the eelworm. In 1953, therefore, advisory entomologists made a preliminary pilot survey on about 100 fields of oats which were known to be infested, in order to decide on the best methods for use in a large-scale random survey. Each field was examined by a soil sampling method and by root-examination of samples of the plants. The soil sampling method was similar to that used for potato root eelworm (*Heterodera rostochiensis*). Each plant sample consisted of a 4- inch (10 cm.) length of row lifted from the soil to include as much of the roots as possible. Each sample was first examined dry by visual inspection, having shaken off loose soil. Samples on which cysts were detected were not examined further. Those which appeared to be free were gently washed in water and examined more carefully. When washing gave a negative result, pieces of the root were stained in 0.05% acid fuchsin/lactophenol and examined under the microscope in lactophenol. The methods were compared for their efficiency in detecting the presence of *H. major* and for the time taken. The conclusion was that the examination of 250 grams of soil based on 25 borings from a field was roughly equal in efficiency to the dry inspection of the roots of 20 samples of oat plants. The examination of two 250 gram samples of soil based on 50 borings from a field was roughly equivalent to dry inspection of the plant samples followed by washing of the roots. The additional information obtained by staining roots was not sufficient to compensate for the considerable extra time involved. In addition, observations were made on the appearance of the root system. Abnormal branching of the roots is known to be characteristic of *H. major* infestation. However, the 1953 investigations indicated that a normal root system was not a safe indication of the absence of cysts but abnormal branching of roots did generally indicate that cysts were present — in other words, obvious root-proliferation was not such a sensitive criterion as the presence of cysts.

NATIONAL SURVEY, 1954

During the spring and summer of 1954 all provincial centres of the National Agricultural Advisory Service took part in a random survey for cereal root eelworm (SOUTHEY, 1955). Advisory entomologists were asked to examine one field growing oats on each of a list of farms already randomly selected for a survey of rabbit damage to winter cereals. Some additional fields were also sampled.

Methods

Observers had the choice of two methods which had been tested in the 1953 pilot survey, namely root examination of samples of plants or soil sampling; some used both methods. In the plant sampling method, roots of the twenty samples were first inspected dry, either in the field or in the laboratory. If cysts were not found in this way the samples were carefully washed and examined again. This examination was done from June onwards when cysts were expected to be visible on the roots.

Soil sampling was done during the first six months of the year to detect cysts already present in the soil before cropping; no attempt was made to recover new cysts formed by the crop. Fifty borings were taken from each field and the air-dried soil examined in the laboratory by the usual flotation method, in most cases by means of a Fenwick apparatus. *H. major* cysts were identified by larval measurement. Viable *H. major* cysts were taken to be those containing larvae in the *H. major* length range (540-580 μ).

Systematic sampling was used with both methods, i.e. the samples were distributed more or less evenly over each field in the form of a grid. Observers were asked to record the date of sowing, the soil type and the degree of damage, if any, to the crop. Standard categories were used for the soil-type and damage records.

Regional Distribution

As a result of the survey, *Heterodera major* is now known to be very widely distributed in England and Wales, even in districts where crop damage has not been experienced. It seems likely that it should be regarded as an indigenous nematode. It is now known to occur in all except five counties, two of which were not examined. 367 fields were sampled, of which 262 were on the randomly selected farms; for the most part only the random sample has been considered in examining the data. *Heterodera major* was found in 113 out of the 262 fields, i.e. 43 per cent. The districts with high proportions of infested fields were not always those where the most serious damage occurred. The incidence of crop damage by cereal root eelworm probably depends much more on local conditions, especially cropping practice and soil type, than on mere presence of the eelworm.

Effect of Soil Type on Infestation

Those who have had practical experience of cereal root eelworm

trouble have claimed that, like potato root eelworm, it is more common and more severe on light sandy soils than on heavy soils. The results of the survey have supported this view.

TABLE I

Cereal Root Eelworm and Soil Type

Soil Type	Total Fields	Fields Infested per cent	Plant Sampling: Total Fields found infested	Plant Sampling: Mean infested plant samples out of 20	Soil Sampling: Total Fields found infested	Soil Sampling: Mean log (n + 1) viable cysts per 500 g.
Heavy [1])	107	35	27	5.3	16	0.73
Medium	66	35	13	6.5	8	0.71
Light	63	60	28	9.0	14	0.93

Table I shows that a higher proportion of infested fields and heavier infestations occurred on the light soils than on the medium and heavy ones. It is possible that results would be influenced by the fact that cysts are more difficult to find on plants from heavy clay soils but in this case one would not expect to find the difference which was observed between the light soils and the medium ones, or the similarity between the results for the medium and heavy soils.

Crop Damage in Relation to Infestation and Soil Type

The observers found it difficult to assess crop damage due to cereal root eelworm because of the various other factors, e.g. other pests and diseases, soil conditions and fertility, which influence the condition of the crop. In many cases it was impossible to estimate with certainty the effect due to cereal root eelworm. For this reason, and because of the small number of fields, it was not possible with any confidence to relate the degree of damage to the level of infestation. However, table II shows, as would be expected, that the proportion of fields in which cereal root eelworm was detected was much greater for those fields classed as damaged by eelworm than for those showing no damage.

Damaged fields also showed on average a higher level of infestation as measured by both plant sampling and soil sampling methods. Eelworm damage observed in the survey varied from a slight check in growth to more or less severe patchiness. In the random sample of fields only one was recorded as being a total failure due to eelworm.

1) Heavy = clay and clay-loam; Medium = loam and silt-loam; Light = sandy loam and sand.

TABLE II

Crop Damage in Relation to Infestation

	Plant Sampling			Soil Sampling		
	Total Fields	% Fields Infested	Mean infested plant samples out of 20 for infested fields	Total Fields	% Fields Infested	Mean log (n + 1) viable cyst count for infested fields
No Damage	116	26	5.3	60	27	0.70 (4 per 500 g.)
Damage	46	89	8.9	22	82	1.04 (10 per 500 g.)

The data suggested that crop damage was more evident on light soils than on heavier ones. For the heavy soils the proportion of damaged fields was 15/75 (20 per cent), for the medium soils, 7/52 (13 per cent) and for the light soils, 18/53 (34 per cent).

Spring Sowing versus Winter Sowing

There was no evidence from the survey results that the sowing season, whether winter or spring, had any influence on cereal root eelworm infestation of oats. 48 per cent of the spring-sown crops were infested and 50 per cent of the wintersown crops. There was no evidence of any difference in average levels of infestation for the two times of sowing.

Efficiency of Sampling Methods

Both plant sampling and soil sampling methods were used on a number of the fields and this provided valuable additional data on the relative efficiency of the methods. For this purpose, I have considered all the fields for which results were available, and not only the random sample.

TABLE III

Relative Efficiency of Sampling Methods for Detection

(Figures are numbers of fields)

		Soil Sampling		
		Detected	Failed to detect	Totals
Plant Sampling	Detected	43	17	60
	Failed to detect	3	31	34
	Totals	46	48	94

Table III shows that soil sampling failed to detect cysts in 17 fields where they were found by plant sampling and plant sampling failed in only three fields known to be infested by soil sampling. Assuming that *Heterodera major* was in fact present in all of the 31 fields in which both methods failed, then soil sampling failed in $17 + 31 = 48$ cases and plant sampling failed in $3 + 31 = 34$ cases out of the 94 fields examined by both methods. It appears that plant sampling was rather more efficient than soil sampling for detecting cereal root eelworm infestations. The data were further used to calculate a regression coefficient for the percentage of plant samples infested on population of viable cysts in the soil. By plotting the plant sampling data against the soil sampling data and drawing the regression line, it was shown that on average the two methods of measuring infestation were related but that for any one field there could be wide variation from this average relationship.

Out of 187 fields examined by the plant sampling method, cereal root eelworm was found by dry inspection of the roots in 82. Of the 105 fields which therefore gave negative results by this method, the eelworm was only found in a further seven after washing the roots in the laboratory. It was concluded that the extra work involved in washing the samples was not justified by the small amount of extra information obtained.

The survey has given us an outline picture of the position over the country and has also provided useful information on methods. The detail is now being filled in by intensive local survey work by entomologists of the National Agricultural Advisory Service, who are also making further studies of important practical problems, such as the effects of different crop rotations and variation in host susceptibility.

Mr. B. M. Church of the Rothamsted Statistical Department was responsible for assessing the results of the 1953 pilot survey and also assisted me in examining the data from the 1954 survey.

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DISCUSSION

B. A. Cooper: Mr. Southey took the history of *Heterodera major* in Britain back to 1908. I think we can go back rather further than that: I have recently been looking at *H. major* cysts from arable land and what I take to be cysts of this species from grassland in many districts.

The arable land cysts are almost entirely thick-walled and heavily pigmented, while cysts from old grassland are mainly thin-walled, with a small proportion of thick-walled types.

(With reference to later comments by Dr. Oostenbrink, I should add that I base my recognition of *major* on cysts with conspicuous bullae placed close to the vulval fenestra, and not displaced anteriorly by a bulla-free area as in *schachtii, trifolii, galeopsidis* and *„limonii"*, with a short bridge (about half the fenestra length) bearing a very short vulval slit, and that I can distinguish these from all described *Heterodera* species and from several undescribed ones).

In soils from Yorkshire, Lincolnshire and Norfolk which have never been cultivated, this preponderance of thin-walled cysts holds good; but in some areas which have been grass since the last century, but were arable before then, the *major* type contain an abnormally high proportion of thick-walled cysts; I take this as evidence that Cereal Root Eelworm occurred here when this land last grew cereals.

Now much of this light, infertile (the farmer would say: "hungry") land has a history, over the centuries, of periods of arable cultivation alternating with periods of neglect or grassland. Economists will say that the land was cultivated only when prices were high enough to pay; I suggest that Cereal Root Eelworm, resulting in bad harvests, may have caused this alternation of husbandry. Certainly much of the land in East Anglia, which is now showing Cereal Root Eelworm damage, can be seen, by an examination of parish records, to have alternated thus. I have little doubt that this pest has been with us for hundreds, perhaps thousands, of years. As Mr. Southey has pointed out, the wide distribution of cases detected by the Survey points to its having been long established in the country, if not indigenous.

Another matter I would like to comment on: Mr. Southey displayed a table which purported to show that "plant-sampling" was somewhat more effective in detecting the species than "soil-sampling". I disagree that this is so.

Separation of cysts in the survey was done by the normal Fenwick-flotation method. Mr. Hesling's paper has already shown that this is likely to miss some of the cysts most full of living contents. Yet diagnosis of the species *major* in the survey was based solely on the measurement of living larvae; cysts containing dead and empty eggs or larvae were rejected. My experience with random field samples is that a high proportion of *H. major* cysts are non-viable. A study of the morphological characteristics of the cyst-wall would have enabled a very much higher proportion of cases to have been recognised from soil samples.

Moreover, in the survey, only 500 grammes of soil were examined from each field sampled. In my laboratory at Kirton I have been using a vibratory separator, which enables much larger samples to be examined quickly. This also would improve the precision of the soil-samplingtechnique by comparison with direct plant examination.

H. Goffart: In Germany cultivation of winter oats is not usual, but we have observed in experiments that winter oats are not damaged to the same extent as summer oats.

J. F. Southey: In England, reports from different districts have been con-

flicting on this point. In some areas it is thought that winter oats may be, if anything, worse affected. However, the survey results suggest that on average, there is no significant difference between winter- and spring-sown crops.

D. ROBERTSON: Has it been definitely proved that *H. major* is not prevalent in N. Ireland?

J. F. SOUTHEY: So far as I know, *H. major* has not yet been discovered in N. Ireland. (After this discussion Mr. Chamberlain from Belfast confirmed that it had not been found in N. Ireland up to that time).

H. DEN OUDEN: Is there a correlation between heavy infestation of light soils and crop rotation with many cereals? (Compare with Beet-eelworm, which occurs on soils with high pH; beets are grown only on high-pH soils.)

J. F. SOUTHEY: This may to some extent account for the prevalence of the eelworm on light soils; crop rotation is certainly one of the most important of the factors which influence infestation. However, it does not seem to be the only reason because it has been observed that local differences in eelworm-attack within a field have in some cases corresponded with local differences in soil type.

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BEITRÄGE ZUM PROBLEM DER AKTIVIERUNG DER HETERODERA-ZYSTEN

VON

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Seit Baunacke (1932) wissen wir, dass das Schlüpfen der Heterodera-Larven durch von den Wurzeln der Wirtspflanzen ausgehende chemische Reize stimuliert wird, dass der wirksame Stoff wasserlöslich, und dass er daher im Wurzeldiffusat enthalten ist. Deshalb wird seit langem zur Gewinnung von Larven für Laboruntersuchungen und zur Prüfung der Wirkung von Bekämpfungsmitteln Wurzeldiffusat benutzt, dessen stimulierender Effekt jedoch von dem physikalischen und chemischen Bodenzustand, von dem physiologischen Zustand der Pflanzen, von der Jahreszeit und von anderen Faktoren abhängig ist, so dass ein Stimulans angestrebt wird, das in seiner Wirkung eine gewisse Konstanz zeigt. Für *Heterodera rostochiensis* Woll. glaubte ich in der Pikrinsäure (0.01 bis 0,05%ig), die bereits von Fenwick (1943) als wirksam erkannt wurde, einen solchen Stoff gefunden zu haben (Nolte, 1955). Bei Fortführung der Versuche zeigten sich jedoch unterschiedliche Ergebnisse je nach Herkunft der Zysten.

Dies veranlasste mich, anstelle der Pikrinsäure Anhydrotetronsäure [1]) zu verwenden, die nach Calam und Mitarbeitern (1949) und nach Johnson (1952), dem wirksamen Stoff des Wurzeldiffusats der Kartoffeln und Tomaten chemisch nahe steht. Die seit Anfang dieses Jahres laufenden Versuche bestätigen die von den Genannten berichtete stimulierende Wirkung und gaben wichtige Anhaltspunkte für Laborprüfungen mit diesem Chemikal.

Im Vergleich zu Wurzeldiffusat wurde, wie an anderer Stelle ausführlicher berichtet werden wird, festgestellt, dass

1) Anhydrotetronsäure auch in den Wintermonaten stimuliert,

2) eine 0,2%ige Lösung besonders wirksam ist,

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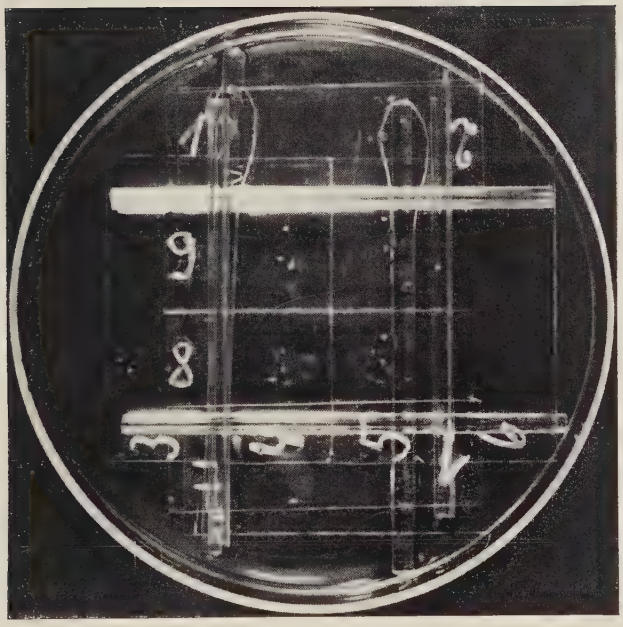
1) Herrn Prof. Dr. Fürst, Dresden, danke ich für die Überlassung der Anhydrotetronsäure auch an dieser Stelle.



Aufbewahrung der Objektträger in einer Petrischale.

3) jedoch eine 0,1%ige Lösung der Wirkung des Kartoffelwurzeldiffusats nahe kommt.

Besondere Bedeutung kommt dem pH-Wert der Anhydrotetronsäurelösungen zu. CALAM und Mitarbeiter (1949) haben mitgeteilt, dass das Schlüpfen bei Anhydrotetronsäure-Stimulierung in pH-Bereichen unter 3 erfolgt, während bei Wurzeldiffusat der wirksame pH-Bereich zwischen 3 und 8 liegt. Bei Prüfung der pH-Werte der verwendeten Konzentrationen konnten diese Ergebnisse von CALAM und Mitarbeitern im wesentlichen bestätigt werden, und es zeigte sich darüber hinaus, dass der wirksame pH-Bereich bei Anhydrotetronsäure-Stimulierung verhältnismässig eng begrenzt ist.

Um eine Beeinflussung des pH-Wertes zu verhindern, wurden die für diese Versuche verwendeten lufttrockenen und bei Zimmertemperatur aufbewahrten Zysten nicht, wie allgemein üblich, vor Zuführung der zu prüfenden Lösung in Wasser vorgequollen, sondern lufttrocken in die Anhydrotetronsäurekonzentration eingelegt. Sie stammten von der Versuchsfläche unseres Institutes in Jerichow (Bezirk Magdeburg). Für jeden Versuch wurden 100 Zysten verwendet, jeweils 5 Zysten wurden auf einem Objektträger mit einem Tropfen der zu prüfenden Flüssigkeit bedeckt; jeder Objektträger wurde mit 2 Tropfen versehen. Um das Austrocknen der Flüssigkeitstropfen zu verhindern, wurden die Objektträger in geschlossene Petrischalen von 14 cm Durchmesser eingelegt (Taf. X), die für die Versuchsdauer in einem dunklen Raum bei 23°C aufbewahrt wurden.

Bei diesen Untersuchungen zeigt sich eine deutliche Abhängigkeit der Schlüpfrate von der Konzentration und vom pH-Wert. Wie Tabelle I erkennen lässt, stimuliert auch noch die 0,02%ige Anhydrotetron-

TABELLE I

Stimulierende Wirkung der Anhydrotetronsäure in verschiedener Konzentration

Konzentration in %	pH-Wert	Zahl der in 20 Tagen aus 100 Zysten geschlüpften Larven
0,1	2,7	1035
0,09	2,82	1068
0,08	2,91	884
0,06	2,99	893
0,05	3,21	255
0,04	3,27	207
0,02	3,30	156
0,01	3,71	22
0,001	5,95	50

säure, ein deutlicher Unterschied in der Wirkung ist aber bereits zwischen 0,06 und 0,05%iger Konzentration zu erkennen. Der pH-Wert der 0.05%igen Lösung wurde in diesem Fall mit 3,21 festgestellt.

Den Einfluss der Konzentration zeigt die Tabelle II mit Ergebnissen von Anhydrotetronsäurelösungen verschiedener Konzentration aber ungefähr gleichem pH-Wert. Die Überlegenheit der 0,2%igen Lösung kommt dabei deutlich zum Ausdruck.

TABELLE II

Stimulierende Wirkung verschiedener Konzentrationen der Anhydrotetronsäure bei annähernd gleichem pH-Wert

Konzentration in %	pH-Wert	Zahl der in 8 Tagen aus 100 Zysten geschlüpften Larven
0,1	2,70	923
0,2	2,68	2209
0,5	2,72	854
0,1	3,19	1157
0,05	3,21	678

Dass jedoch für die gleiche Konzentration dem pH-Wert Bedeutung zukommt, lässt die Tabelle III erkennen. Die hier gezeigten Unterschiede traten in allen Wiederholungen gleichsinnig auf.

Für die praktische Verwendung der Anhydrotetronsäure ergibt sich

TABELLE III

Stimulierende Wirkung gleicher Konzentrationen der Anhydrotetronsäure bei verschiedenem pH-Wert

Konzentration in %	pH-Wert	Zahl der in 8 Tagen aus 100 Zysten geschlüpften Larven
0,5	2,52	479
	2,72	854
0,2	2,68	2209
	2,85	1828
0,1	2,70	923
	3,19	1157
0,09	2,82	864
	3,27	353
0,05	3,21	678
	3,84	340

daraus die Folgerung, dass für vergleichende Versuche nicht nur die Konzentration, sondern der pH-Wert der betreffenden Lösung berücksichtigt werden müssen, und dass schon geringfügige Schwankungen des pH-Wertes das Ergebnis beeinflussen können.

Zusammenfassend kann gefolgert werden, dass Anhydrotetronsäure in Konzentrationen von 0,02 bis 0,2% wirksam ist, und dass der wirksamste stimulierende Effekt im pH-Bereich 2,5 bis 3,2 erzielt wird.

Die Versuche mit Anhydrotetronsäure dienten der Vorbereitung für umfangreiche Untersuchungen über eine Beeinflussung des Schlüpfens der Kartoffelnematodenlarven durch Antibiotika.

Antibiotika beeinflussen bekanntlich den Stoffwechsel niederer Organismen und werden daher mit Erfolg in der Human- und Veterinärmedizin angewendet. Sie fördern bei Zugabe zum Futter die Entwicklung von Haustieren. Für die Phytopathologie ist von Bedeutung, dass zahlreiche Antibiotika gegen pflanzenparasitische Pilze und Bakterien wirksam sind, und dass solche auch schon mit Erfolg gegen Schadinsekten geprüft wurden (Klinkowski 1954, Köhler 1950 und 1953, Nolte 1955). In allen Fällen spielt eine Direktwirkung auf pflanzliche Mikroorganismen eine Rolle. Auch für die Förderung der Haustiere und die Abtötung von Insekten wird eine solche angenommen, und zwar für die Haustiere eine Beeinflussung über die Darmflora, für die Insekten eine Beeinträchtigung über die Endosymbionten. Für die Insekten konnte nun jedoch Beck (1950) zeigen, dass auch eine direkte Beeinflussung des Oxydase-Systems erfolgen kann und die oxydativen Vorgänge gestört werden.

Wir wissen seit langem, dass sauerstoffabspaltende Chemikalien das Schlüpfen von Heterodera-Larven stimulieren. Nach Doliva (1954) handelt es sich dabei jedoch nicht um eine direkte Aktivierung der in der Eihülle ruhenden Larve durch eine Sauerstoffzufuhr, sondern um eine Wirkung des Sauerstoffs im status nascendi und um eine oxydative Aufspaltung der Proteine der Eihülle, die dadurch an Festigkeit verliert, dem Druck der im Innern zusammengerollt liegenden Larve nachgibt und diese frei lässt. Da dieses Freiwerden der Larve passiv geschieht, lehnt die Verfasserin die Bezeichnung „Aktivierung" für den Vorgang ab, vergisst jedoch dabei, dass wir unter „Aktivierung der Heterodera-Larven" nicht nur die Befreiung der Larve aus dem Ei sondern gleichzeitig das Schlüpfen aus der Zyste verstehen, dass sich die Stimulierung auf beide Vorgänge erstrecken muss, und dass im Verlauf dieses Vorgangs physiologische Veränderungen in der Larve vor sich gehen, die sich in unterschiedlicher Reaktion der

eigentlichen Eilarve und der endgültig aktivierten Larve auf physikalische und auf chemische Reize zeigen. Der Ausdruck „Aktivierung" hat also bei Betrachtung des Gesamtvorgangs seine Berechtigung.

Wenn nach Beck (1950) Antibiotika die oxydativen Prozesse im tierischen Körper beeinflussen können, darf auch eine Wirkung auf das Schlüpfen der *Heterodera rostochiensis*-Larven aus der Zyste durch Beeinflussung der oxydativen Aufspaltung der Eimembran angenommen werden. Um diese Frage zu prüfen, wurden von mir bisher 124 Kulturfiltrate [2]) von 50 Stämmen verschiedener gegen Bakterien oder Pilze wirksamer Penicillium-Arten und Actinomyceten geprüft.

Die zu prüfenden Zysten wurden zunächst für 1 oder 2 Stunden in die Kulturfiltrate getaucht, abgespült und dann in 0,1%ige Anhydrotetronsäure überführt. Einige Ergebnisse wurden in der Tabelle IV zusammengestellt.

TABELLE IV

Schlüpfrate nach Antibiotika-Behandlung und Stimulierung mit Anhydrotetronsäure

Kulturfiltrat:		Zahl der in 20 Tagen aus 100 Zysten geschlüpften Larven
I. Penicillium-Arten		
Stamm-Nr.	9	812
	28	926
	15	1015
	42	1134
	1912/1	1135
	17	1258
	11	1742
	12 b	1863
	9 a	1900
II. Actinomyceten		
Stamm-Nr.	14/5	484
	204/2 c	531
	1011/2 b	676
	5	807
	gris 6	837
	2610/13	844
	1011/23	989
	304/11	1235
III. Kontrolle: Anhydrotetronsäure 0,1 %ig		845

2) Für die Überlassung der Kulturfiltrate danke ich Fräulein Dr. Köhler, Aschersleben, auch an dieser Stelle.

Bisher hat keines der geprüften Kulturfiltrate eine schlüpfhemmende Wirkung gezeigt. Das stimmt mit Ergebnissen, die Mai (1954) mit Kulturfiltraten von Bakterien und verschiedenen Pilzen erzielt hat, überein. Einige der in Tabelle IV wiedergegebenen Zahlen für Kulturfiltrate von Penicillien liegen über der Kontrolle mit Anhydrotetronsäure. Ob jedoch daraus auf eine schlüpffördernde Wirkung dieser Pilze geschlossen werden darf, muss erst noch durch weitere Prüfungen festgestellt werden.

Bisher kann gefolgert werden, dass weder die von Mai noch die von mir geprüften Pilze und Bakterien eine Schlüpfhemmung verursachen, dass einige vielleicht aber stimulieren. Daraufhin jedoch heute schon ein abschliessendes Urteil über eine Bedeutung der Antibiotika zu fällen, halte ich für verfrüht, solange nicht die Prüfung auf breitester Basis und unter Berücksichtigung aller methodischen Möglichkeiten erfolgt ist.

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DISKUSSION

D. W. Fenwick: Questioned that A. T. A. was more active than potato-root-diffusate, since he has found the reverse to be the case. He asked whether Mr. Nolte had examined more than one sample of A.T.A. since he, Dr. Fenwick had found that different samples gave different results. Also questioned the significance of using A.T.A. which worked at pH 2,5 or so while normally potato-root-diffusate was unreliable at this pH.

H. W. Nolte: Meine Ergebnisse mit Anhydrotetronsäure beziehen sich auf Versuche während der Wintermonate. Zu dieser Zeit zeigte sich Anhydrotetronsäure dem Wurzeldiffusat überlegen. Im Sommer ist es umgekehrt. Ich habe nur Anhydrotetronsäure gleicher Herkunft geprüft. Die Verwendung von Anhydrotetronsäure ist zur Gewinnung von Larven und zur Prüfung irgendwie behandelter Zysten gedacht. In letzterem Fall dient als Vergleich Unbehandelt + Anhydrotetronsäure. Ein Vergleich mit Wurzeldiffusat ist daher nicht erforderlich.

B. Homeyer: Ist die geringe Schlüpfzahl nicht weniger durch die Jahreszeit als vielmehr durch die Austrocknung der Zysten zu erklären? Das würde z. T. auch Ihre Behauptung erklären, dass die Oxydationsprozesse in der Zyste das Schlüpfen der Larven regeln.

H. W. Nolte: Zweifellos spielt die Austrocknung eine Rolle. Das zeigen Ergebnisse, die nach Vorwässern der Zysten gewonnen werden. Um jedoch die Prüfung auf die pH-Abhängigkeit nicht zu beeinflussen, musste ich auf das Vorwässern verzichten.

NEMATOLOGICAL NEWS

In 1954 Nematological News was sent for the first time as type-script to some forty colleagues in various countries as a means of communication and help in nematological research. Nematological News will now be continued in this journal and will cover the following topics: discussion of problems in nematode nomenclature and other questions of general interest; citation of recently published papers in all countries; personal news of colleagues such as change of address etc.; inquiries about "missing" papers.

The editor of this appendix of "NEMATOLOGICA" would like to receive as much information as possible regarding the above mentioned topics. Thus, Nematological News may become a valuable help especially for those nematologists who are not able to survey the whole of current literature. Therefore, please send reprints of your own papers to the editor immediately after their publication, and give notice of published works of other authors on plant parasitic, freeliving, freshwater, marine and soil nematodes. If possible, write in English, French or German. — All colleagues will greatly appreciate your trouble.

Arwed H. Meyl, Braunschweig (Germany), Rebenring 45.

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pfähle des Nord-Ostsee-Kanals und der Kieler Förde. — Kieler Meeresforsch. XI: 110-135. (Among other animal groups, 32 species of nematodes are quoted together with short descriptions of their habitats.)

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—— (1955), Factors Influencing the Emergence of Larvae from Cysts of the Beet Eelworm, *Heterodera Schachtii* Schmidt. — J. Helm. XXIX: 3-16. (Temperature optimum was 25°C. Constant temperatures do not provide optimum conditions for larval emergence, a higher rate occurs under fluctuating temperatures. Rate of larval emergence in beet diffusate increased as the initial oxygen concentration increased. Pre-treatment of cysts at low oxygen conc. reduced the subsequent emergence in diffusate. Fluctuating oxygen conc. reduced emergence. Larval emergence was found also to be dependent on saturation deficiency and soil moisture.)

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Change of Address:

JONES, F. G. W. (Lecturer in crop pests) and H. R. WALLACE (Scientific Officer), both of The School of Agriculture, University of Cambridge, now: F. G. W. JONES (Head of Nematology Dept.) and H. R. WALLACE (Scientific Officer), Rothamsted Experimental Station, Harpenden, Herts.

WINSLOW, R. D. *Now:* Science Service Laboratory London, Ontario, Canada.

MORIARTY, F. has been appointed S.O. to replace H. R. WALLACE in Cambridge.

PETERS, B. G. left Rothamsted Experimental Station, Harpenden, to became Professor of Parasitology at Imperial College, London, on Oct. 1st, 1955.

ALLGÉN, CARL. *Now:* Stallmast. gt. 21 C, II, Malmö, Sweden.

A. H. MEYL

(*Nematologica,* Vol. I, 1956, no. 2)

SURVEY OF THE RESULTS OF FOUR YEARS EXPERIMENTS ON THE CHEMICAL CONTROL OF THE POTATO ROOT EELWORM

BY

J. VAN DEN BRANDE, R. H. KIPS AND J. D'HERDE

(Rijkslandbouwhogeschool, Gent)

When the first infestation of potato root eelworm in Belgium was discovered in 1949 (1) it was soon realised that in view of important economic aspects of this particular pest control problem, research work should be started immediately. The available information at the time was not very substantial and there was no practical experience to build on. At first, progress was very slow and the experiments were carried out in a small way. In a first series of experiments, the results of which were published in 1951 (2) various possible nematicides were tested. They included DD-mixture, ethylene dibromide, benzene hexachloride and a xylene emulsion. Before treatment, picric acid was used on some of the plots, as this compound was claimed to induce hatching of larvae from the cysts and might thus increase the degree of control obtained by the various chemicals. Soil sample examination, number of new cysts found on the roots and yield were all used as criteria to assess the results of these experiments. Only DD gave significant increases in yield (300%) and from the numbers of cysts formed on the roots, expressed per gram of dry root weight, it was shown that the treatment had resulted in a percentage kill of about 80%. Soil sample examination post harvest however made it clear that on the whole this outwardly very successful treatment had not succeeded in reducing the eelworm population. The picric acid pre-treatment proved to have been of no value. EDB was eliminated from further research as its phytotoxic effects were pronounced and its nematicidal value negligeable. The assertion that DD treatment resulted in a stimulation or soil amendment effect was also investigated, using tomato as a test plant and the height as criterion. The results proved to our satisfaction that no such soil amendment effect occurred or at least was of very little importance. EDB significantly retarded growth in these experiments.

Calcium cyanamide was tested in a 7 × 7 latin square, using very high dosages, but is was not possible to draw definite conclusions from the experiment. No direct nematicidal effect could be shown, although it was clear that this treatment had some influence on the eelworm population as compared with ammoniumsulfate.

These were the results of our preliminary investigations. A number of points were established, some of which were only confirmations, but the net result was on the whole not very promising. Apart from the fact that a great deal of experience had been gained as regards the technique, an accidental occurrence in the course of these trials was to determine the future line along which research was to proceed and prove to be of great importance.

In the DD experiments part of a treatment had to be carried out in pouring rain. A detailed study of the final figures showed very much better results for all examined criteria. At the time, the importance of this fact was not realised. In a later trial however, in which the effect of the split-application was studied, similar conditions occurred and the differences were so striking, that a detailed examination of this factor humidity became necessary. The variations and even discrepancies which could be found in the literature on the effect of a DD treatment on the potato root eelworm might find a plausible explanation in this direction.

Laboratory experiments were carried out in which presoaked, wetted and dry cysts were exposed to various chemicals including DD, CBP, CP, EDB, Demeton, Schradan, Isopestox. The experiments were carried out at different, constant temperatures nl. 23°C and 6°C. The results of these experiments were very striking. At 23°C dry cysts exposed to DD vapours for one month, still contained viable larvae which could be induced to hatch by exposure to potato root secretion. When using wet cysts a 20 minutes exposure in the same conditions resulted in a 100% kill. For CBP, the most powerful nematicide so far tested, a five day exposure was necessary to kill dry cysts, not even five minutes were necessary when they were wetted. At low temperature (6°C) 8 days were insufficient. All compounds tested showed this very great influence of the humidity condition of the cyst on the nematicidal activity of the chemicals. These results were confirmed in soil experiments, using fairly large wooden boxes. Dry dune sand soil was mixed with water, so as to obtain a number of different water contents, varying from 0,7% (air dry soil) to 18,2% (saturated). The results were conclusive. In air dry soil, at a dose corresponding to

4l/are the number of larvae hatched from all cysts, even those in the immediate vicinity of the injection point, was similar to the controls. A slight addition of water (4,2%) immediately resulted in a 100% kill in all points, except those in the top inch of soil where between 5 and 10% of the larvae remained viable. An optimum was reached at 11,8% where a 100% kill was obtained in all points using the 8l/are dose. At 15,8% the cyst contents were not completely killed in the top inch, whilst at saturation level only the cysts very close to the injection point were influenced by the chemical.

The experiment in air dry soil was repeated using the 8l/are dosis, the box being completely sealed off. After 10 days the cysts were recovered. A strong DD odour was noticed when the box was opened, but the larvae hatched normally from the cysts.

Carrying out a similar experiment at low temperature, similar results were observed, except that a 100% kill was not obtained (99,7%). This result is important considering that in the course of the experiment the temperature varied between + 8° C and — 8° C, which tends to indicate that in practical conditions under which soil fumigation is likely to be carried out, temperature will have but little influence.

Investigating the time of exposure it was found that at room temperature, the distribution of the fumigant in the soil occurred very quickly. After 1 day, at 3% water content, a 100% kill was obtained in all points except the top inch, where between 90 and 97% of the cyst contents were killed. Only after between 4 and 8 days was a 100% kill obtained in all points. An increase in the water content of the soil tended to slow down the distribution but only to a very small degree. At 11%, 2 days were necessary to obtain the same situation as at 3,9% after 1 day. After 8 days, differences were no longer apparent. This rapid distribution is important as it makes it possible to consider the water content of the soil at the time of treatment as a criterion for a successful soil fumigation.

Important conclusions could be drawn from these experiments. First of all it was established beyond doubt that the water content of the soil at the time of treatment will determine the final result of the treatment. Not, as was generally believed only because of its influence on the distribution of the fumigant in the soil, but because of the influence of moisture on the interaction cyst-chemical. In dry dune sand soil, the fumigant is quickly and efficiently distributed, but the

cysts are not killed. Only when the water content of the soil is at a certain level, the range having been determined for dune sand soil, the cysts are killed by the nematicide. Only under extreme wet conditions the water content does influence the distribution of the fumigant, thus preventing an efficient control.

There is no clear cut explanation available of this phenomenon. THORNE in a paper on beet eelworm advocates moist conditions as being ideal for keeping nematodes active, suggesting that in this condition they are more susceptible to the nematicide.

Surely, this can not be the explanation of our experiments as the cysts are only just wetted and are dried after the very short exposure time (5 or 20 minutes). It is doubtful whether the activation of the cysts, which have been kept in a dry state for months in the laboratory, can proceed at such a speed. Also, neither the presoaking time, nor the use of a surface active agent, nor the use of root secretion produce any difference in the influence of moisture on the cyst-chemical interaction.

On the other hand, soaking in water post treatment has a very strong influence on the nematicidal action of DD. When the cysts are dried immediately after being removed from the fumigation chamber an exposure time of 20 minutes is sufficient to obtain a 100% kill. Soaking in water post treatment for three days before drying, necessitates 90 minutes for the same result. This does not seem to be the case with CP and CBP for the exposure times tested. This seems to indicate that a certain amount of DD is retained by the cyst which can be removed by soaking but not by drying and continues its lethal action after removal from the fumigation chamber.

Important also from a practical point of view are the results of the experiments at low temperatures. With DD, soil temperature does not seem to affect very much its nematicidal action, as long as the temperature does not fall below freezing point. Although the distribution may proceed more slowly, the final result will not be greatly influenced as long as the water content of the soil falls within the limits indicated.

An important latin square field trial confirmed these results. Very thorough measures were taken to prevent reinfestation of the plots and the treatment proceeded very carefully, the fumigant being introduced in the soil by hand through a long tailed funnel. Applications were made when the soil temperature was low (2,5°C) and when it was relatively high (16,5° C). Two different levels of soil moisture were

compared under the latter conditions nl. air dry top soil, with an average water content over a depth of 20 cm of 7%, and moist soil (12.5%). Rates of 4l and 8l/are were used.

The results obtained in hatching experiments on cysts recovered from soil samples taken 20 days after treatment show an important difference in nematicidal action between dry as compared to moist soil. In the former case, treatment at 16,5°C results in a 53,6% or a 87,2% kill according to the dose, whilst in moist soil a 100% kill is obtained with both concentrations. The counts of new cysts formed on the roots gave similar results: under ideal conditions on the average only 0,5% of the cysts present on the control plant were counted, over 40% in dry soil. Under favourable moisture conditions yields were 50% higher than on the plots with dry top soil treated with the same dose, and more than 17 times greater than the yields on the control plots. No significant difference was found between the treatments at the two temperature levels as far as the criteria under consideration are concerned.

The fact that only the top inch or so of the soil was dry at the time of treatment and that in the experimental fields just behind the dune lines, reinfestation through the action of the very strong winds proved impossible to prevent, enhances the importance of the differences observed and thus provides excellent confirmation of the laboratory observations.

A rather interesting example of an application of stressing the importance of the humidity factor in the control of potato root eelworm is afforded by the working out of a method of killing the cysts adhering to flower bulbs of tubers. Begonia and Gloxinia tubers were treated with emulsions of a number of volatile fumigants. Cysts were added and killed as expected during the anticipated times of exposure. But only with CBP at concentrations of 0,05% and 0,025% was a 100% kill obtained without damaging the tubers. A very efficient, simple and practical method was thus devised in a very short time.

SUMMARY

A large number of experiments were carried out with several nematicides or compounds thought to influence the development of the potato root eelworm. They include dichloropropane-dichloropropene, chloropicrin, ethylene dibromide, chloro-bromopropene, demeton, schradan and calciumcyanamide.

It has been shown, especially using DD-mixture, that the moisture condition of the cysts at the time of treatment largely determines the nematicidal effect of the chemical. Soil moisture at the time of treatment should be considered

in this respect, and not merely be taken into consideration when waterlogging prevents the distribution of the fumigant into the soil.

In dune-sand soil, 100% kill was obtained in laboratory experiments at a concentration of 8 l/are between 4,2 and 11,8% water content of the soil, working at room temperature. The optimum for this soil type lies between 10 and 12%.

Although in experiments where the temperature ranged between +8°C and —8°C, it was not possible to obtain a 100% kill, it is thought that in practical working conditions for soil fumigation, the influence of soil temperature is of little importance.

Field experiments confirmed these results. Treatment under ideal moisture conditions in the summer gave a 100 % control when based on soil sample examination and 99,6% when based on counting new cysts on the roots. Under dry conditions these figures were respectively 53,6% and 74,6%. Treatment during the winter gave no appreciable difference, except for the fact that nowhere was a 100% kill obtained. Yields per plant under ideal conditions varied from 1700 gr per plant to 96 gr per plant in the controls.

It is thought that these considerations might prove helpful to explain certain discrepancies in the results of various published experiments on the control of the potato root eelworm.

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DISCUSSION

H. R. WALLACE: I should like to know how a "uniformly moist-soil" is obtained, since when soil is wetted from the top there is not a uniform distribution of water throughout the soil. The water advances in a front with a high moisture content behind the front and a low moisture content before the front.

THE AUTHORS: Perhaps the term "uniformly moist-soil" may be misleading to some extent. The only implication meant is that, when taking soil samples over a depth of 8 inches for water content measurements, there is no apparent difference in the outward appearance of the soil over the entire depth,

and that there is no significant difference in the results obtained (% water) from the different samples taken on one plot.

Perhaps it is also important to note in this connection that all the work reported has been carried out on dune-sand soil.

To wet the soil it was sprinkled as evenly as possible by means of a watering-can.

H. GOFFART: I would like to ask if you have observed differences on the increase of nematode populations after treatment especially if the increase is higher in fields with 100% control as in fields with 50% mortality.

THE AUTHORS: This question has not been investigated.

L. N. STANILAND: I have been working with liquid nematicides in the form of solubilized chemicals (in particular xylenols solubilised with detergents of alkyl sulphate type). The action of these liquid nematicides give the highest control when soil and contained cysts are fairly dry. When soil is injected with DD fumigant it is in the top 2 or 3 inches, where soil and cysts are dry and where there is insufficient concentration of the fumigant, that there is only a poor control of potato root eelworm. This being so, it seemed that a practical method would be to combine the two treatments. This has been tested under laboratory and field conditions and gives control which is nearly complete. Each of the two treatments does what the other does not and so they work together. The soil is first injected with DD (400 lb/acre) and then watered with 2 gallons per square yard of the liquid nematicide. This is economic for use in glasshouses and is now being used by some growers in England. It is not economic for use on land in the open, except to treat small infested areas or where a very valuable crop is being grown.

(*Nematologica*, Vol. I, 1956, no. 2)

THE RESISTANCE OF POTATOES TO ROOT EELWORM

BY

T. D. WILLIAMS

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The search for resistant potatoes has been necessitated by the lack of economically suitable control measures against potato root eelworm. The susceptibility of all European potato varieties may be ascribed to the loss of many genes for disease resistance early in the history of European potato breeding.

Resistance to potato root eelworm was found in some of the material held by the Commonwealth Potato Collection (ELLENBY, 1954a). Selfed seed from these plants was grown at Cambridge and further screening carried out by Dr. H. W. Howard of the Plant Breeding Institute and F. G. W. Jones (JONES, 1954). Resistant lines selected from the seedlings of *Solanum tuberosum* sub-species *andigenum* and *Solanum vernei* provide most of the material used in the research at Cambridge.

In this discussion, the term 'resistance' denotes the greatly reduced rate of reproduction of the parasite on resistant potato plants. 'Resistance' is not used here to imply absence of injury in plants invaded by the eelworm.

The criteria of nematode resistance in animals (LAPAGE, 1937) also apply to eelworm resistance in potatoes. In the case of the potato root eelworm, an additional factor operates, the 'hatching factor' contained in potato root diffusate. If this is absent, the rate of hatching of the eggs is much reduced. Consideration of the influence of the 'hatching factor' makes it possible to place plants in the following categories (JONES, 1954, slightly modified):

1. Absolutely resistant. Not invaded, due to root structure or biochemical properties of the root and its exudate.

2. Partially resistant. Invaded.

(a) Reduced larval invasion. Less effective structural barrier or low diffusate activity. The reduced invasion observed in *S. vernei* may result from low diffusate activity.

(b) Considerable larval invasion but larvae may (i) fail to develop, (ii) develop but fail to mature, (iii) larvae mature but females few in number.

3. Susceptible. Large numbers of females produced. High reproduction rate.

The hatching activity of potato root diffusate is assayed by exposing replicated batches of cysts of the potato root eelworm to plant leachings taken under standardised conditions. The rate of larval emergence is used as the index of the potency of the diffusate. Diffusates from a range of resistant plants were compared in this way and it was found that most of the resistant plants tested produced strongly active diffusates. Plants of *Solanum vernei* however, produced a much less active diffusate, as also did plants obtained from a *S. stenotomum* × *S. vernei* cross. These results show that the *S. andigenum* plants tested do not owe their resistant properties to a failure to produce an active diffusate.

Examination of the roots of resistant plants, which have been exposed to infection by potato root eelworm, has shown that all the plants so far studied are invaded by larvae in varying degrees. To enable fairly rapid estimations of invasion to be made, a macerator was used for removing the larvae from the roots. The roots were stained with acid fuchsin (GOODEY, 1951), thus making the larvae easier to count. The macerated roots (of known weight) were passed through a series of sieves (60, 100, and 300 mesh/inch). Corresponding aperture sizes are 0.231, 0.142, and 0.053 mm. respectively. Unwanted debris was removed, and examination of the filtrate showed that more than 90% of the larvae were retained on the two lower sieves.

Several lines of *S. andigenum* and *S. vernei* plants were examined in this way, plants of each type being lifted at regular intervals over a period of a hundred days from the time of emergence of the shoot above the soil. All the plants were grown in pots of soil having the same level of infestation (301 eggs/gm. dry soil).

A distinction was made between second stage 'invasive' larvae and those which had undergone further development, the numbers of each class and the total larval content of the sample of root were plotted graphically against the age of the root. All the results for the lines of *S. andigenum* plants used were considered to be sufficiently similar in pattern to justify their grouping together in one graph. This compensated for the lack of replication resulting from the limited amount of material available.

The resulting graph showed the following features:

1) A steep early rise in the total number of larvae.

2) Preponderance of second stage larvae in this period.

3) The increasing proportion of larvae undergoing further development with increasing time, until the peak was reached.

4) Sharp peak (1600 larvae/gm. of root) at 20-22 days.

5) Fall in number of larvae after 22 days.

The numbers of larvae found in the roots of these resistant plants were as great as those found at a comparable time in susceptible potato plants, although very few mature, or developing females were seen on the resistant plants. Similar observations were made on plants resistant to *Meloidogyne* attack (Barrons, 1939) and also on *Solanum nigrum* plants subjected to potato root eelworm infection (Doncaster, 1953). All the *S. andigenum* plants contained many nearly mature male larvae after 20 days though no counts have yet been made of those which emerge from the root.

The sharp peak in the graph may be compared with that which occurs in the non-cumulative larval hatching curve obtained from cysts placed in diffusate. The peak is the result of the emergence of the 'hatcheable' larvae over a short period of time.

The results of the tests of diffusate activity and studies of larval invasion and development so far carried out, indicate that *S. andigenum* plants must be considered to be partially resistant to potato root eelworm.

References have been made from time to time, to the possibility of variations in the sex ratio of parasitic nematodes (Molz, 1920; Cobb, Steiner & Christie, 1927; Ellenby, 1954b). Changes in the sex ratio might well be determined by food availability. The female of the beet eelworm *Heterodera schachtii* has been estimated to need as much as thirty times as much food to reach maturity as does the male (Sengbusch, 1927). Competition for food materials may occur if many larvae enter the root at one point, when there is a limit to the number of giant cells which the host can produce.

So far, few giant cells have been observed in resistant potato plants and these are always associated with the few females which mature. Further work is in progress to see whether males can mature without giant cells, and also to determine the point at which female development ceases in resistant plants.

There would appear to be two possible explanations of the very small numbers of females occurring on resistant plants. One would be the death within the root of female larvae, the other a change in sex ratio leading to almost all males and very few females. It is hoped to use resistant plants to determine which process is operating. If an

accurate count can be made of males emerging from the roots of resistant plants, and this compared with the number of larvae known to have entered the roots, it may then be possible to decide the process involved. This comparison may be made difficult, however, if there is a high death rate in the root irrespective of sex.

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DISCUSSION

C. Ellenby: I was very interested in the results reported for *S. vernei,* for it seems that it is possible that this species may perhaps be more promising as a source of resistant material than the resistant forms of *S. andigenum*; whereas only a few lines out of over 700 lines of *S. andigenum* were found by me to be resistant, every one of the forms of *S. vernei* I tested was found to be very promising. I think we should be careful that we are backing the right horse in our breeding for resistance.

It is very difficult to assess the validity of tests carried out with root diffusate obtained from different lines for quite often the type of root differs very markedly and, although Fenwick's method of standardising root diffusate is of very great use, it is useless for dealing with qualitative differences between types of plants. For example, I was very interested in the results reported for

1) References quoted from M. T. Franklin, 'The Cyst forming species of *Heterodera*'.

S. vernei in this respect, but from my experience the roots of this species are very different in their general form from those of the domestic and many of the South American potatoes.

It is very tempting to explain everything in terms of a shifting sex ratio; the danger about my findings on the shifting sex ratio is that they seem to explain almost everything. But I think that if the organism is capable of developing into a male or a female depending on environmental conditions, this process must be of an anticipatory nature; there would be no point at all in having a mechanism which only came into operation when, in fact, due to the concentration of females, there was no possibility of providing them with adequate nourishment. While on this subject, I should like to say that I have certain evidence for the existence of differences between varieties of the domestic potato in the manifestation of the shifting sex ratio and, in view of Dr. TOXOPEUS' remarks [1]), that I have some experiments proceeding at the moment which I hope will demonstrate whether the potato root eelworm is capable of parthenogenesis.

I do not think we should be alarmed at the possible development of new biotypes of the eelworm, capable of development to maturity on the resistant potatoes. This development must be expected. But the position with a soil borne disease is very different from that of an air borne disease. For example, the problem of obtaining blight resistance is considered by some plant breeders to be insuperable because it is an air borne disease and the new biotypes of the blight organism soon spread; on the other hand, wart resistant potatoes are still of great use in spite of the existence of new biotypes of the wart organism. The potato eelworm being soil borne should behave like wart disease, provided that we behave ourselves in our cropping. It also is important that we should not concentrate on a single line of resistance; several lines will make the evolution of new eelworm biotypes more difficult.

I should be grateful if Mr. WILLIAMS would enlarge on two points mentioned in his resume:

1) Whether, when giant cells are found, this is true of all members of a clone, or only of some of them.

2) Whether, in his reference to the lack of development of larvae in *S. vernei*, he means that this is always the case. In 1948 I found odd cysts on a very resistant line of this species, as I have already noted in publication.

T. D. WILLIAMS: I must apologise for not discussing the results of examinations of *Solanum vernei* roots invaded by *Heterodera rostochiensis* larvae. Although mentioned in the summary of my paper I omitted them to keep within the time available. However, I am glad of this opportunity to present them in more detail.

In the *S. vernei* material used in 1954, the highest number of larval stages found in the root was 300/gm. and this peak occurred when the shoots had been above ground for 50 days. This contrasts with the peak number of 1600/gm. of root found at 20 days in *S. andigenum*. Whereas in *S. andigenum* plants large numbers of almost mature male larvae, and many adult males were seen, no such development was seen in *S. vernei*.

Further work in 1955 confirms these observations, since roots of plants which were 50 days old contained only one or two larvae per gram which had entered the third stage. A different clone of *S. vernei* has been used this year and the highest number of larvae per gram of root so far recorded was 700 but as in the previous year, the number of larvae showing development past the 2nd. stage was very small. No fourth stage or mature males have been seen, nor any

1) See pages 100-101.

developing or mature females. I have not seen any cysts on *Solanum vernei* roots but I have found cysts and 'giant cells' on the roots of plants from a *S. vernei* × *S. stenotomum* cross. These observations can I think be taken to indicate a high degree of resistance in *Solanum vernei,* though whether it is suitable material for breeding purposes I cannot say.

To the question about the occurrence of 'giant cells' in the whole of a clone I can only answer that just one or two pieces of root from a few plants were examined for 'giant cells'. In all cases, where cysts were seen, these cells were found also. I cannot say whether this would be true for all the members of any particular clone.

BREEDING FOR RESISTANCE TO THE POTATO ROOT EELWORM IN THE NETHERLANDS

BY

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(Foundation for Agricultural Plant Breeding, Wageningen)

INTRODUCTION

In the Netherlands the search for resistance to the potato root eelworm began in 1946. In England Dr C. ELLENBY had already been working on this problem since 1941, viz. by a systematic examination of the Commonwealth Potato Collection at Cambridge (ELLENBY 1945). He found resistance in *S. ballsii* and according to a later publication (ELLENBY 1952), as early as 1948 he had obtained a clue of resistance in 5 clones of *S. andigenum*, viz. the numbers C.P.C. 1673, 1685, 1692, 1595 and 1647.

In the Netherlands work was done with crosses of *S. ballsii* C.P.C. 105 and *S. sucrense* C.P.C. 162 at that time, however without significant results.

HISTORICAL SURVEY

In January 1951 Dr H. J. TOXOPEUS, senior breeder at the Institute of Agricultural Plant Breeding, Wageningen, who was then engaged in this work, received some seeds from 3 of the 5 *andigenum*-clones mentioned above, viz. the numbers C.P.C. 1673, 1685 and 1692.

In the same period a research team (Research Team for Control of the Potato Root Eelworm) was formed with the aim of investigating the possibilities of potato root eelworm control and as a consequence the breeding work could be extended considerably.

Dr TOXOPEUS was responsible for the work in connection with breeding and the present writer, a fellow-worker of the Foundation for Agricultural Plant Breeding (Stichting voor Plantenveredeling, S.V.P.), Wageningen, was appointed to assist him in this work.

The above-mentioned seeds were sown immediately after receipt. Later cuttings were made from the resulting seedlings. The rooted cuttings were planted in pots of 16 cm diameter, which were filled with thoroughly mixed, heavily infested soil. Results were visible after about two months:

All the plants derived from a certain seedling were either crowded with cysts or they were completely or almost completely free from cysts. Our data and those of Dr ELLENBY were published in a joint report (ELLENBY 1952; TOXOPEUS & HUIJSMAN 1952).

Prior to and even after this discovery we were working with hybrids of the wild species *S. ballsii* and *S. sucrense*. The seedlings derived from the crosses with these wild species were still extremely wild, their degree of resistance to *Heterodera* was low and the mode of inheritance of the resistance was not clear.

However, after receipt of the resistant material of *S. andigenum*, the outlook for the breeding of resistant potato varieties had become much better. In the first place because resistance had been found in a cultivated species from Peru, which is closely related to *S. tuberosum*. Secondly because the difference between the susceptible and the resistant varieties was so clearly defined. It appeared to be easy to classify the seedlings as resistant or susceptible owing to lack of transitory forms. Unfortunately, it was known too late in the season of 1951 which were the resistant seedlings. Therefore the crossing work had to be postponed. However, in the summer of 1952 crosses could be made on a large scale with the material that had proved to be resistant. The resistant *andigenum*-seedlings were intercrossed and also crosses were made between resistant *andigenum*-seedlings and our susceptible potato-varieties.

During the winter of 1952/53 some hundreds of Fi seedlings were raised in a glasshouse in pots with heavily infected soil for a study of the mechanism of inheritance. The outcome of this work justified a large-scale sowing in March.

It could be established that the resistance is dominant. It also appeared that the seedlings from the cross *andigenum* × *tuberosum* raised under artificial light, presented no difficulties in classification and this enabled us to set up a breeding programme, which was amply sponsored by financial aid from the above-mentioned Research Team and the Foundation for Agricultural Plant Breeding.

BREEDING WORK

During the summers of 1953 and 1954, numerous seedlings were tested for resistance. This material was derived from intercrosses of resistant *andigenum*-seedlings as well as from crosses between resistant *andigenum*-seedlings and susceptible *tuberosum*-varieties.

Testing for resistance was performed in the following way: the

seedlings were raised in pots of 8 cm diameter with soil free from cysts. When sufficiently rooted they were transplanted into pots of 16 cm diameter, filled with heavily infected soil. These pots were dug in on a trial field which was specially chosen for the purpose on a very isolated place. After 6-8 weeks the rootballs were removed from the pots in order to examine them on cysts. For this purpose only the surface roots of the balls were inspected. The examination took place at a time when most cysts were bright yellow to orange in colour.

It appeared that the seedlings could easily be grouped into two classes, viz. a group with heavily attacked roots and a group with roots which were completely free from cysts or which showed only a few cysts.

The ratio found between the resistant and the susceptible plants could be explained with sufficient evidence by assuming that the resistance was due to a dominant gene H. This monogenic dominance is somewhat complicated on account of the fact that *S. andigenum* and *S. tuberosum* show tetraploid segregations.

Resistant seedlings can be of the following genotypes.

Hhhh: simplex; gives 50% resistant offspring when crossed with a susceptible variety (hhhh = nulliplex)

HHhh: duplex; gives 83% resistant offspring when crossed with a nulliplex variety

HHHh: triplex; gives 100% resistant offspring when crossed with a nulliplex variety

HHHH: quadruplex; gives 100% resistant offspring when crossed with a nulliplex variety

When the resistant seedlings with the least effective genetical constitution—the simplex seedlings—are crossed with a susceptible potato variety they yet produce a progeny half of which is resistant.

Therefore it is easy to raise large numbers of resistant seedlings. Resistance alone, however, is not sufficient and has to be combined with the commercial characters of our existing potato varieties. The breeding of such varieties is a very arduous task.

On the basis of the knowledge of the inheritance, it was possible to set up an adequate breeding programme. For this purpose we used the method of repeated backcrossing.

In order to obtain practical results within a short time, the Dutch potato breeders have been involved in the breeding work at a very early stage. In the Netherlands there are now some 200 potato breeders, working on a widely divergent scale. All have a lot of ex-

perience and are highly skilled in potato growing and breeding. In the spring of 1954 the Foundation for Agricultural Plant Breeding placed at their disposal about 80000 true seeds, derived from crosses between resistant *andigenum-tuberosum* hybrids and commercial varieties—the first backcross. In the spring of 1955 about 120000 seeds of the same generation of a far better quality than those of 1954, were distributed. The first generation hybrids used as resistant parents had been selected more intensively for commercial characters.

As the growing of potatoes on infested soil is statutory prohibited in the Netherlands, the breeders themselves cannot test their selected seedlings for resistance. This is done for them at the Plant Protection Service at Wageningen, during the winter.

BROADENING THE BASIS OF THE BREEDING WORK

In order to broaden the basis of the breeding work new material is continually searched, for resistance. In 1950 it was already known that resistance occurred in *S.ballsii* and *S. sucrense*.

In the following years we also found resistance in seedlings of *S. famatinae, S. macolae* and in the *andigenum*-clone Potozi 7 from Bolivia.

Dr Toxopeus, who made a trip to Peru in the early part of 1955 succeeded in collecting several hundreds of samples of cultivated potatoes occurring in Peru, Bolivia and in the Argentine Republic. Some few resistant clones may be expected to occur in this material.

NATURE OF THE RESISTANCE

The nature of the resistance in the above-mentioned material and also in *S. andigenum* C.P.C. 1673, 1685 and 1692 always appeared to be similar. All resistant plants produce a root diffusate containing the hatching factor and when they are grown in infested soil, numerous larvae invade the roots.

Resistant seedlings of the clone C.P.C. 1673, which was most thoroughly examined, were invaded by the same number of larvae as the susceptible sisterplants. The larvae penetrate into the roots in the second stage and most of them do not develop any further.

However, some manage to reach a more advanced stage of development and most of these appear to be of the male sex; only rarely mature or nearly mature females are produced. It could not be established whether or not these males are sexually mature.

Differences could be stated in the number of resistant seedlings

with isolated cysts on the roots, in sowings derived from different basic material. This number is more or less a measure for the degree of resistance. We found that resistant material derived from C.P.C. 1673 has very few of such plants, those derived from C.P.C. 1685 some more and the degree of resistance of Potozi 7 was so low, that among the resistant seedlings of crosses between this clone and susceptible varieties, not a single plant occurred that was entirely free from cysts (Toxopeus & Huijsman 1953).

The nature of the few mature cysts on the roots of resistant plants deserves some consideration. One can suppose that there are no genetic differences in the larvae, but that a few grow to maturity, owing to favourable internal and external conditions. Another possibility is that these cysts are genetically different from the population and consequently are able to complete their sexual cycle. It is obvious that from such genotypes physiological races may develop, that break down the resistance of our breeding material. So far the results of experiments to settle this question indicate that these cysts are not genotypically different from the majority of the cysts.

Nevertheless one has to assume the possibility of the development of new physiological races sooner or later and for this reason it is necessary to search for new material with a different genetical background, i.e. to broaden the basis of the breeding programme as much as possible.

The resistance of *S. andigenum* C.P.C. 1673 prevents the building up of a population of cysts in the soil. From our trials it has appeared that as a consequence of growing resistant plants a considerable decrease in the number of viable eggs per weight unit of soil occurs. This decrease is of the same level or even larger than the one which occurs when crops are grown that are not attacked by the potato root eelworm.

On extremely heavily infested soil the very large number of invading larvae causes severe damage to the resistant crop, which is also manifest in a yield reduction.

The nature of the resistance is still unknown. It may be a substance in the roots that inhibits the development of the larvae, but it is also possible that some substance necessary for the normal development of the larvae is lacking. In this connection we made an experiment by grafting resistant plants on susceptible ones and the other way round. The plants reacted completely according to the genotype of the rootstock variety. No influence of the scion could be stated. The results of

this experiment do not allow of any conclusions as to the nature of the resistance. A better knowledge about this nature would be very important, particularly in connection with the chemical control of the potato root eelworm.

SUMMARY

A review of research and breeding work on resistance to the potato root eelworm (*Heterodera rostochiensis*) is given. Though in the Netherlands a start with this work was made in 1946, it lasted until 1951 before it could be developed successfully, owing to the discovery of the resistance in *S. andigenum.*

Breeding work was relatively simple on account of a clearly defined difference between resistant and susceptible seedlings. Resistance proved to be dominant. Some complications arose because *S. tuberosum* as well as *S. andigenum* are tetraploid.

Introduction of resistant genes is easy, but combining resistance with other desirable features is a very arduous task. For the execution of the breeding programme the method of repeated backcrossing is used.

Private breeders were involved by a large-scale distribution of seeds derived from crosses with resistant seedlings. The nature of the resistance is discussed.

No positive indications of the occurrence of physiological races of the eelworm have been found, but the assumption of such a possibility makes the broadening of the basis for resistance desirable.

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(*Nematologica*, Vol. I, 1956, no. 2)

SOME REMARKS ON THE DEVELOPMENT OF NEW BIOTYPES IN HETERODERA ROSTOCHIENSIS THAT MIGHT ATTACK RESISTANT POTATO-CLONES

BY

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In the fore-going paper on breeding for resistance to *Heterodera rostochiensis* Mr. HUIJSMAN tells us that, on resistant plants, females succeed only in forming cysts in isolated instances and that these are poor in structure and contain only a few eggs. These cysts are probably not different genotypically from those which did not attain maturity. It is therefore highly improbable that these females could give rise to new biotypes.

However, as with all parasites, one may expect that when in the future large fields are planted with resistant potato-varieties, new biotypes of *Heterodera rostochiensis* may be selected out of the population by the resistant plants.

At some time a very rare cyst filled with viable eggs may develop on a root of a resistant plant and it is worth considering the probability of a dangerous biotype developing from such a female cyst. This question touches the core of the problem of the reproduction of *H. rostochiensis*, its breeding system.

We have to know if fertilization of the eggs has to precede the development of larvae and if so, whether the males leaving the roots of a resistant plant are sexually mature. It is also important to know if the ability of the larvae of the new biotype to develop into mature females in the roots of a resistant plant is a dominant character or a recessive one.

If fertilization is not necessary and the larvae can develop parthenogenetically from the eggs, the population can build itself up rapidly; however, if fertilization is obligate, the new biotype may have difficulty in maintaining itself.

Conditio sine qua non for fertilization of the eggs is that the males leaving the roots of the resistant plants are sexually mature. If the formation of fertile spermatozoids has not taken place, fertilization of the

eggs, and consequently reproduction, is impossible. Some of the males may be of the same biotype as the female in question and such males may be fertile and able to mate with her, at least if they leave the root sufficiently near. Though the chance of such a mating is extremely low, it is possible, and it could be the beginning of a successful, though probably slow development.

However, a male of the new biotype need not be fertile as the gene(s) controlling the fertility of females may be different from those governing the maturation of the male.

If all males leaving the roots of the resistant plant are fertile, the female of the new biotype will mate with the old biotype male and only very exceptionally with a male of its own type which has to compete with an overwhelming majority of old type males.

If the ability of the female larvae to develop into a mature female in the roots of a resistant plant is a dominant character, the new biotype has a ready change to come to the fore. If, however, this character behaves as a recessive one none of the hybrid larvae will have a chance of reaching maturity and no building up of the new race can take place.

This survey of what can happen certainly is not complete; however, I think it proves that it is very difficult to estimate the chances of the development of populations of *Heterodera rostochiensis* that are able to break down the resistance of the potato-varieties we are breeding now.

For the time being the breeder has to be prepared for the worst and I want to stress the necessity of continuing the search for more resistant material with a different genetical background as a safeguard for future unfavourable developments.

Though there are many workers devoting themselves to the study of nematodes, the number of those working on questions concerning reproduction seems to be small. It is the aim of the above remarks to direct the attention of the specialists to the importance of such studies for our breeding work, for only as a result of such studies can we have any understanding of the problem of the possible development of new biotypes of nematodes in general and especially of *Heterodera rostochiensis*.

(*Nematologica*, Vol. I, 1956, no. 2)

INHERITANCE OF RESISTANCE TO STEM NEMATODES IN RED CLOVER

BY

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In several European countries the stem nematode, *Ditylenchus dipsaci* (KÜHN) FILIPJEV, is a dangerous parasite in red clover and sometimes in white clover and lucerne, too. Studies of the distribution of nematode attacks in Sweden (BINGEFORS, 1950) showed that serious damage was caused by the parasite in all farming areas in the country. Possibly, some agricultural districts far to the north were exceptions. However, nematode attacks have been found in red clover leys up to a latitude of 66°.

In southern Sweden the attacks have been known for some 50 years and they have been followed—by natural selection—of an improvement of the resistance in local red clover strains. But they have also led to a real breeding program in order to develop highly resistant red clover strains. In central and northern Sweden the attacks have not occurred for a very long time and local strains from these parts of the country are as a rule very susceptible. However, in these susceptible strains, too, breeding is able to improve the resistance very much, as I hope to show later on. When breeding programs are planned it is important to know the inheritance of resistance. In connection with other nematode investigations at the Ultuna branch station of the Swedish Seed Association this problem has been studied for some years. Results which are presented here are preliminary but may contribute a little to make clear the inheritance.

Inheritance of resistance to stem nematodes in red clover has not been directly studied as far as I know from the literature. However, FRANDSEN (1951) found that crosses between susceptible and resistant red clover strains could sometimes give progenies with very high resistance. In lucerne BURKART (1937) showed that resistance to stem nematodes was inherited in a complicated way.

The inheritance of resistance in different kinds of plants has been studied for some other nematode species. NILSSON-EHLE's (1920)

investigations of resistance to *Heterodera major* in barley are the most comprehensive. He showed that the resistance with all probability was determined by one dominant gene.

It has been shown that some American *Solanum* species are resistant to attacks by *Heterodera rostochiensis*. TOXOPEUS and HUIJSMAN (1952 and 1953) found in crosses between *Solanum tuberosum* and *S. tuberosum* var. *andigenum* that the resistance in some progenies was determined by one dominant gene with tetrasomic inheritance. In other progenies they had to presume the occurrence of two genes, causing resistance when both were present. JONES (1954) also studied the inheritance of resistance in similar crosses.

GOFFART and ROSS (1954) showed that resistance to *Heterodera rostochiensis* in *Solanum vernei* probably was determined by several genes.

The inheritance of resistance against *Meloidogyne incognita* and *M. incognita* var. *acrita* in *Lespedeza stipulacea* was studied by HANSON, ROBINSON and WELLS (1954). Crosses between susceptible and resistant plants gave very variable susceptibility in the progeny families. Both parent types were found, too. An estimate of heritability showed that very good improvement could be expected if the selection was carried out according to family averages for resistance.

Red clover breeding at Ultuna has aimed at selection of nematode resistant plants in susceptible but well adapted local strains, mainly the Ultuna strain. This strain is very susceptible to nematode attacks and shows less than one per cent resistant plants when artificially infected in the laboratory and with the number of plants without nematodes determined ten weeks after infection. As a comparison it may be mentioned that the south Swedish strain Merkur gives between 80 and 90 per cent resistant plants. By intense selection for two generations the resistance in the Ultuna strain improved very much. In the fall of 1945 plants were selected in a severely damaged red clover ley and seed was harvested from individual plants in 1946. All the plant progenies were tested for resistance in the laboratory and a number of plants of each family were planted in a nematode infested field in 1947. From the best families seed was harvested in 1949. When the seed had been multiplied resistance was determined by laboratory tests. Expressed as indices of resistance where the degree of multiplication of the nematodes was taken into consideration, the Ultuna strain gave 19 $\pm$ 0,2 while Merkur gave 92 $\pm$ 1,7. The new selection, called U 056, gave 68 $\pm$ 5,5. U 056 has been tested in a

number of field trials, too. Most of them were performed in nematode infested fields. In ten trials in 1st year leys and five trials in 2 nd year leys the following mean relative values were obtained:

	1st year ley	2nd year ley
Ultuna	*100*	*100*
U 056	135	211
Merkur	141	186

Thus, it is clear that by selection of the most resistant plants in a susceptible red clover strain it is possible to obtain valuable improvements in a very short time.

In order to study the inheritance more in detail, crosses were made between plants belonging to the two strains Merkur and Ultuna and to a population with these two strains as parents. The resistance of the parents was determined by infection of older plants grown in pots. These plants were cut down and when they started to grow again nematodes were placed between the young leaves. The air around the plants was kept saturated with moisture for a week after infection. Partly, these determinations were carried out on plants from which seed had been harvested, but cuttings of plants used or intended for crosses were infected, too. The resistance of the progenies was determined by infection of seedlings. A drop of a nematode suspension was placed between the cotyledons. The infection was repeated twice and the air around the plants was kept saturated with moisture. The development of the attacks in individual plants was determined ten weeks after infection. Plants were killed in boiling acetone and treated with osmic acid according to Godfrey (1935). The occurrence of plants with and without nematodes at this time was determined.

When estimating the results it is important to know whether the determinations of resistance after infection of seedlings and the determinations after infection of older plants agree. It has been shown that determinations in older plants may be somewhat unreliable in a few instances, especially if no cuttings have been infected. As a rule the agreement is good, however. It was also possible to compare determinations of 6 to 15 plants from each of a number of progenies which had been infected when they were one year old and plants from the same progenies infected as seedlings. From each family 50 to 100 seedlings were tested. An estimate of the correlation gave $r = 0{,}7983$ with $t^2 = 15{,}81$ ($P < 0{,}01$). Thus, there is a satisfactory agreement beween these two ways of determining the resistance.

Crosses between susceptible plants, between resistant plants and

between resistant and susceptible plants were performed and analysed. In most crosses plants from the population Ultuna x Merkur were pollinated by susceptible and resistant Merkur plants. Reciprocal crosses were performed to some extent. Plants from the very susceptible Ultuna strain were crossed with Merkur plants and with plants from Ultuna x Merkur. As examples of crosses between susceptible plants results from 15 crosses between Ultuna x Merkur plants and Merkur plants may be given. In 10 of these crosses none or very few resistant plants were obtained. In 5 crosses some more resistant plants were found. Thus, one cross gave 40 per cent resistant plants and four crosses 10 to 20 per cent such plants.

In all 39 crosses between resistant Ultuna x Merkur plants and resistant Merkur plants were carried out and analysed. In all these progenies a high number of resistant plants were found and the average for all the progenies was 82 per cent. Estimate of the heterogenity shows, however, that the crosses do not represent a single type of segregation. While 19 progenies gave between 85 and 90 per cent resistant plants and 7 progenies between 80 and 85 per cent, there are only very few crosses with a lower number of resistant plants. Especially noticeable is the fact that not a single cross gave more than 95 per cent resistant plants and that only 6 progenies had between 90 and 95 per cent such plants.

Most tested progenies had been obtained by crossing susceptible and resistant plants. The segregation in such crosses is very divergent and an estimate of the heterogenity shows that the values obtained do not represent a single type of segregation. A few crosses between a susceptible Ultuna plant and resistant Merkur and Ultuna x Merkur plants gave very divergent segregations.

In all 67 crosses between susceptible and resistant plants were performed and analysed. One progeny was very susceptible and gave only 2 per cent resistant plants and 5 progenies gave 10-25 per cent such plants. Nine progenies gave between 25 and 35 per cent resistant plants and 18 progenies gave 40-50 per cent such plants. Still more progenies, 23, belong to a group with 60 to 70 per cent resistant plants. Between these three groups there are a few crosses. Two crosses gave more than 70 per cent and not a single one more than 75 per cent resistant plants.

Results from all these crosses have been brought together in table I and in fig. 1.

It is interesting to compare the results obtained by BURKART (1937)

TABLE I

Number of progenies in classes with different percentages of resistant plants.

Per cent resistant plants	Cross: susceptible x susceptible	resistant x resistant	resistant x susceptible and reciprocal	All the crosses
0-5	9		1	10
5-10	1			1
10-15	2		1	3
15-20	1		2	3
20-25	1		2	3
25-30			5	5
30-35			4	4
35-40			2	2
40-45	1	1	8	10
45-50			10	10
50-55			2	2
55-60			5	5
60-65		1	9	10
65-70		1	14	15
70-75		2	2	4
75-80		2		2
80-85		7		7
85-90		19		19
90-95		6		6
95-100				
In all	15	39	67	121

in lucerne and these results in red clover. In BURKART's investigations 71 studied F_1-plants from three crosses between susceptible plants gave no quite immune plants and one plant with a low degree of resistance. In red clover we have tested 730 plants from 15 crosses between susceptible plants. Five progenies gave some resistant plants and in all 81 resistant plants were found.

BURKART studied 149 plants from four crosses between resistant and susceptible plants and found 89 resistant plants, that is 61 per cent. Variations between 47 and 65 per cent occurred. In red clover we have tested 4755 plants and 2371, 50 per cent, showed resistance. However the variations are large. There seems to be a group of progenies with about 30 per cent, another group with about 45 per cent and a third group with about 65 per cent resistant plants.

BURKART also studied 76 plants from five crosses between resistant plants and of these 61, that is 86 per cent, were resistant. In red clover we analysed 39 crosses with 1990 plants. Of these 1639, 82 per cent, were resistant. BURKART found variations between 67 and 91 per

cent while the variations in red clover were from 41 to 95 per cent.

When we compare these two species, red clover and lucerne, we must remember that in lucerne tetrasomic inheritance may be expected. However, such inheritance has been found only very seldom. BURKART found his results to show that the resistance is dominant over susceptibility but that the resistance is not dependent on only one gene. Neither do simple schemes for two or three genes explain the inheritance. It is clear that the inheritance of resistance in red clover is complicated, too. However, the rapid selection effect in a susceptible strain

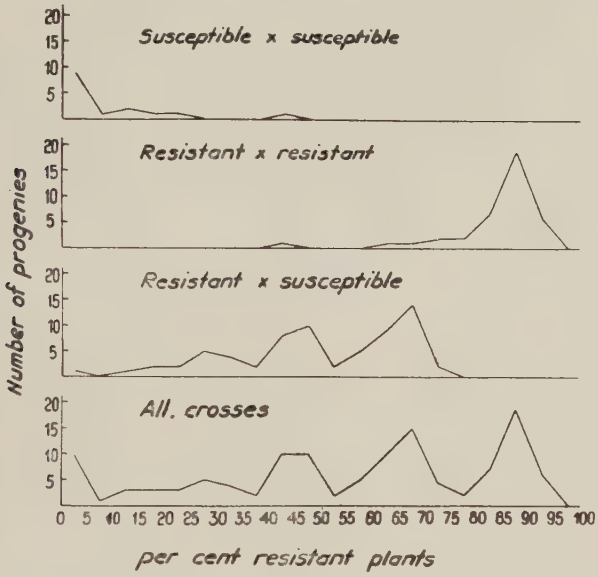

Fig. 1.

as well as the possibly uneven distribution of the progenies regarding the occurrence of resistant plants may indicate that a few genes have a major influence.

In order to obtain more information about the inheritance, continued investigations were planned to analyse backcrosses to the resistant as well as to the susceptible parent. However, it was difficult to keep susceptible parent plants alive. Not until this year has it been possible to perform backcrosses to the susceptible parents to any greater extent. However, many backcrosses to resistant parents have been made. These backcrosses seem to give very complicated results and up to now we have not analysed a sufficient number of crosses to be able to throw more light upon the inheritance of resistance.

These investigations have been supported by grants from the Royal Swedish Academy of Agriculture.

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DISCUSSION

L. SIMON: Bei der Überprüfung der Anfälligkeit gegen *Ditylenchus dipsaci* von süddeutschen Rotkleesorten ergab sich, das auch die Herkunft der Nematoden eine Rolle spielt.

So würden die Rotkleesorten von Nematoden norddeutsches Herkunft weniger (etwa 50%) befallen als von Nematoden süddeutsches Herkunft. Ich möchte Dr. Bingefors fragen, ob in Schweden ähnliche Beobachtungen gemacht würden.

S. BINGEFORS: We have found some differences between nematodes from different parts of Sweden. However, the differences are not very great. It may be that stem nematodes have spread in a rather short time to different parts of Sweden and that there are relatively small differences between the nematode populations because of this fact.

(*Nematologica*, Vol. I, 1956, no. 2)

SOIL POPULATION STUDIES USING MICROPLOTS

BY

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(School of Agriculture, University of Cambridge)

Experiments with microplots were undertaken to overcome some of the difficulties associated with population studies using field plots, such as lack of control over cultivations and over within-plot variability in soil structure and eelworm population (JONES 1955 a, b). A summary of some of the results of microplot work with beet eelworm (*Heterodera schachtii* SCHM.) is given below. Cruciferae caused greater increases than Chenopodiaceae when cultivated at low initial population levels, and greater decreases at high initial levels. Non-hosts and inefficient hosts caused reductions in population levels of the same order as efficient hosts in the same plant families. When sugar beet was grown at varying initial population levels, the final populations tended to rise to a 'ceiling' which fluctuated from season to season. The 'ceiling' effect also appears to occur in the field and has been observed by GOFFART (1952) in pot tests. It may be used to compare the efficiency of different hosts or, if the same host is grown, to measure the effect of different soils and different seasons. A linear relationship exists between the logarithm of the initial population level and the yield of roots and tops of sugar beet. The main effect of eelworm attack upon sugar beet is to modify the size of the plant without greatly affecting sugar metabolism. Heavily attacked plants are unable to take advantage of favourable growing conditions and the difference between lightly and heavily attacked plants is sharpened by seasons favourable to the growth of sugar beet. Within certain limits the final populations produced after the cultivation of a host are independent of yield or plant population density. Cruciferae are far less susceptible to injury by root invasion than the cultivated varieties of *Beta vulgaris* L. It is suggested that host efficiency in raising eelworm populations and susceptibility to injury are distinct and independent attributes of host plants. Decay of eelworm population in Chatteris soil in land under fallow or under non-host plants in non-host families, is of the order 20%, 40% and 50% per annum for cysts, cysts with contents and eggs respectively, and appears to be largely independent of the initial population level. The average content of 'cysts-with-contents' drops from 177 eggs/cyst in the year after cultivating a host crop, to 131, 118 and 114 in the second, third and fourth years.

The cultivation of crops influences the *in vitro* hatch from beet eelworm cysts in the following spring. Low hatches are obtained after host crops, especially where there has been considerable increase. A period of 'maturation' appears to be necessary before the eggs contained in newly formed cysts are fully sensitive to hatching stimuli.

A full account of the above work is published in the Annals of applied Biology (JONES, 1956).

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DISCUSSION

J. H. SCHUURMANS STEKHOVEN: What is the meaning of the sentence: "The cultivation of crops influences the *in vitro* hatch from beet eelworm cysts in the next spring?

F. G. W. JONES: If cysts are taken from fallow soil they give an *in vitro* hatch in root diffusate of 80% of their contents, while cysts taken after sugar beet or other hosts give a low hatch, perhaps as low as 30% after great increase.

D. W. FENWICK: asked whether he had any information on the degree of cyst-emptying prior to new cyst-formation.

F. G. W. JONES: None. but I presume that low hatches after host crops are due to two processes: 1) The emptying of old cysts by root diffusate action. 2) The production of new cysts in which the eggs require a period of maturation before they are fully sensitive to hatching stimuli.

D. W. FENWICK: suggested that the "ceiling" stipulated by Mr. Jones was possible evidence of some mechanism within the plant which limited the number of adult females within the roots.

D. ROBERTSON: asked what factors prevent the eelworm increasing to the stage when the plants were completely destroyed?

F. G. W. JONES: The relationship between size plant and the initial number of eelworms in the soil is not linear but logarithmic. I suggest that the main factor limiting increase is intraspecific competition within rootlets.

H. DEN OUDEN: How many borings are taken in sampling microplots?

F. G. W. JONES: About 20 per plot after the crop has been lifted and the soil dug over to destroy any local effects due to the position of the plants.

E. ELLENBY: Although it would be a mistake to consider that only a single factor is involved in the control of the reproductive rate, I feel fairly confident that a variable sex-ratio is at least one of the factors.

F. G. W. JONES: It is not yet known whether this also occurs in beet eelworm. Another limiting effect is "root-pruning". If the attack is sufficiently heavy the root may die near the plant and cause the death of any immature forms more distally placed.

(*Nematologica*, Vol. I, 1956, no. 2)

A SOURCE OF THE VARIATION OF HATCHING DATA FOR THE POTATO ROOT EELWORM, HETERODERA ROSTOCHIENSIS WOLLENWEBER

BY

C. ELLENBY

(Newcastle-on-Tyne, England)

A full account of this paper will appear in the Annals of applied Biology.

SUMMARY

Cysts of the potato root eelworm were treated in a number of ways. After a preliminary soaking, some were cut in half, some treated with trypsin, and some treated with trypsin and then cut in half. Emergence of larvae from lots of 25 of these cysts was then investigated.

Emergence was highest from the halved cysts; but perhaps of greater interest was the fact that marked differences in variability were also found. The coefficients of variation for the halved cysts and the halved trypsin treated cysts were a fraction of those for the intact cysts. The results are discussed in relation to the conception of the cyst as a unit.

DISCUSSION

B. A. Cooper: I should like to ask how Dr. Ellenby's cysts were obtained: were they old cysts or young cysts or a mixture of young and old?

Dr. Bovien mentioned yesterday that fresh cysts are "unfenestrated", the hatching window at the vulval end of the cyst has not yet opened. As the cysts get older, the natural hatching hole — the "fenestra" — has opened. Later still the neck-aperture becomes open for hatching, and mechanically produced holes also serve as hatching pores.

Even cysts of the same age are visually variable, in that some have a membrane covering this fenestra. Trypsin is possibly a substance which assists fenestration, which would explain the great variation in dealing with uncut cysts.

C. Ellenby: The cysts used in my experiments were all of the same age, about a year. My results on variability certainly suggest the possibility of the existence of physical differences between cysts and I am therefore very interested in Dr. Cooper's observations. But, for reasons it would be too difficult to summarise briefly, I am confident that fenestration alone is not a sufficient explanation for my findings.

B. Homeyer: Auf dem 2. Bilde fiel auf, dass die Zahl der geschlüpften Larven nach 8 Tagen sehr stark abnahm. Würde die Trypsin-Lösung gewechselt oder welche Gründe hat es?

C. Ellenby: The various Trypsin-treated cysts were treated with the enzyme only *before* they were stimulated with root-diffusate. During the hatching experiment fresh solutions of diffusate were applied every fifth day until emergence had effectively ceased. The fall referred to by Dr. Homeyer is therefore not connected with any change of treatment during the hatching experiment and is probably due to the very heavy emergence at the beginning.

(*Nematologica*, Vol. I, 1956, no. 2)

THE INFLUENCE OF ORGANIC MANURING ON THE DEVELOPMENT OF THE POTATO ROOT EELWORM, HETERODERA ROSTOCHIENSIS

BY

P. A. VAN DER LAAN

(T.N.O.; Laboratory of Phytopathology, Wageningen)

INTRODUCTION

It has been known for several years that organic manuring influences the development of cyst nematodes. Investigations on the beet cyst eelworm, *Heterodera schachtii*, mainly carried out in Germany, lead to the conclusion that an extra manure application increases the tolerance of the plants against this disease and that especially organic manuring had a much better effect in this respect than artificial fertilizer.

The effect of green manuring was already found by REINMUTH in 1929, whilst results on the influence of farmyard manure were given by MILES c.s. in 1943. Yields of four different potato varieties were doubled after receiving a mixture of combined farmyard manure and artificial fertilizer. The number of cysts in the soil had increased only 45%.

Similarly THOMPSON (1949), GOFFART (1949), OOSTENBRINK (1950) and SCHMIDT (1953) mention this relatively favourable effect of farmyard manure, basing their opinions on many years of experimentation. In all cases, plots having received farmyard manure had less eelworm cysts and higher yields than those plots treated with artificial fertilizers. However, the manuring with compost resulted in the presence of a very large number of cysts in the soil and potato sickness was soon found to occur in these plots (OOSTENBRINK). Since compost applications stimulate root formation and since more roots mean more food for the nematodes, we looked for an explanation in this direction.

In all these experiments the eelworm population was calculated yearly by counting the in- or decrease of cysts containing viable larvae. Yields from these test plots were determined.

However, plant parasitic nematodes, such as the potato root eelworm are obligate parasites. Investigations on such parasites are only

promising if both the parasite and the host are surveyed. Therefore counts of cysts present in the soil before and after harvest may lead to false conclusions if the growth of the host is ignored. Yield results of harvested potato-tubers may help, but diseased potato plants do not show an exact correlation between the amount of roots (seat of parasite) and the number and weight of resulting potato tubers. This problem is best shown by the following example.

It was frequently shown in experiments with the potato root eelworm, that after killing 90 percent of the eelworm cysts by chemical treatment, the increase of cysts after the harvest was greater in the treated, than in the untreated plots. In the latter, many roots contained only partly developed eelworms which starved due to potato sickness developing, and hence lack of food. Thus most of the eelworm larvae caused their own death, with the result that these plants showed only few new cysts. In the treated plots however, the surviving 10 percent of cysts had multiplied enormously on the apparently healthy plants.

It has therefore become important to link our findings with a botanical investigation of the host. The quantity of root system development is a most important factor in this research. This point was perfectly emphasized by CHITWOOD and FELDMESSER (1948). MILES, however, in 1930 counted already numbers of nematodes, present in 5 mm pieces of potato root!

The influence of organic manure is not restricted to cyst nematodes: recently, OOSTENBRINK (1954) found that the number of meadow nematodes (*Pratylenchus spec.*) on various crops, was significantly lower in farmyard manured plots than in these to which fertilizer had been applied. Some of OOSTENBRINK's figures are summarized in table I.

Not enough is known about the biological background of this problem. In 1938 an explanation was attempted by LINFORD c.s. based on his experiments with *Meloidogyne spec.* in Hawaii. Diseased ananas plants were cut into small pieces which were placed into large glass vessels and allowed to decay for 12 weeks. One part of organic matter to 24 parts of soil was used. Similar vessels did not receive this organic matter. This period is sufficiently long to achieve complete decay under tropical conditions. During this period numerous observations were made on nematode numbers, mites and fungi developing in these vessels. In vessels containing organic matter LINFORD found many saprozoitic nematodes (*Cephalobus, Dorylaimus, Aphelenchoides*). He was also able to identify 4 species of nematode-catching fungi. Larvae

TABLE I

Influence of farmyard manure on the numerical increase of meadow nematode (after OOSTENBRINK *1954)*

Number of eelworms per 200 cc of soil (I and III) and per 10 g of roots (II and IV)

			Pratylenchus
I	Spring 1953	A	236
		A + F	228
II	July 1953	A	5672
		A + F	2551
III	Spring 1954	A	*1326*
		A + F	*602*
IV	June 1954	A	*18.815*
		A + F	*11.155*

A = Artificial fertilizer, A + F = Artificial fertilizer + Farmyard manure: mean of 12 plots with different crops.

of *Meloidogyne* were rare. The glass containers of series two which had not received organic matter, contained less saprozoitic nematodes and fungi, and in one experiment out of five more *Meloidogyne* specimens.

LINFORD continued his work with extension trials. Soil from both series (soil + organic matter and soil only) was placed in pots and sown with cow-pea (*Vigna sinensis)* which was allowed to grow for 35 days; the roots were then examined for the typical knots formed due to *Meloidogyne*. Very large differences could be found between the two tested series. Series A which had received organic matter was practically free from root knot nematode infestation. Plants grown in soil only, showed a very heavy attack by *Meloidogyne*. These experiments were replicated several times with similar results. Pots which had been aged for the first trial were, after the removal of the cow-peas planted with ananas. Once again, growth in the organic manured pots was better with far less nematode attack than in the "soil only" series.

The authors then drew the following conclusion: During the first 12 weeks of the experiment the number of saprophytic and saprozoitic organisms in the organic manured series had increased enormously. Consequently the *Meloidogyne* larvae starved through lack of food in

this period. However, the number of their natural ennemies, such as other nematodes (*Mononchidae* and *Dorylaimidae*) as well as fungi catching nematodes was numerous. The explanation for the favourable effect of organic manuring should therefore be sought in biological control.

METHODS, (OTHER WORKERS)

The development of *Heterodera* and *Meloidogyne* larvae in the roots can be traced by the use of various methods (CHITWOOD and FELDMESSER, l.c.). Nematodes in the roots are stainable with lactophenol-cotton blue (GOODEY 1951). Their numbers may then be determined and separated according to their different stages of development. Counts will then be given for the number of larvae per 5 mm of food (MILES). The roots were coloured with osmium acid by OTEIFA (1953). Five stages in the development of *Meloidogyne* could be noted and counting stopped after one hundred larvae (of all types) had been counted. This method gives a good idea of differences in the development rate of the various series. In some instances 90 first stage larvae and 10 second stage larvae were found, at other times 50 first, 30 second and 10 third stage larvae were counted. However, the total number of larvae per root system or per gram of root could not be determined. The drawback of these staining methods is, that they are very time-consuming.

Several methods are based on the numerical determination of newly formed females ("white cysts"), which are clearly visible on the roots. TRIFFIT (1929) counted these white cysts which could be seen on the visible roots of a potplant when removed from its pot. CHITWOOD and BUHRER (1945) determined the number of white cysts which one person can count in one minute. CHITWOOD and FELDMESSER, as also FENWICK and REID (1951) used a method whereby the whole root-system was washed and weighted. The white cysts are released in the water and the addition of a saturated solution of $MgSO_4$ resulted in a homogenous suspension of cysts in water and enabled the cysts samples to be counted. However, the disadvantage of these methods is, that only a single larval stage, namely the adult female, is counted. As the sex ratio varies (ELLENBY 1954), the number of females counted is certainly not the most exact standard to establish infestation rate. Moreover differences in the rate of development cannot be traced by counting the white cysts only, as these may develop quicker and therefore appear much earlier in one series than in the other.

AUTHOR'S METHOD

The following method was used in our investigation. Since the number of larvae, present in the roots is dependent on the amount of root available, we invariably determined the number of larvae present in one gram of root.

It should be better to determine nematode numbers per square cm of root surface, but this is not practical except with very young plants.

Our experiments were confined to pots in which the complete root-system of one potato plant can be harvested at any required time. Trials were started with series of about 40 pots each and we investigated the root-systems of six pot plant from each series, each at different ages. The complete root-system of each plant was carefully harvested, cleaned by spraying with tap water and so freed from adhering particles. It was then superficially dried and weighed. The resulting roots were then cut into one cm. pieces. About 250 cc tap water was added and the suspension milled for one minute in a Waring Blendor [1]). The suspension was then sieved through a 100 mesh sieve. Nematodes and root particles do not pass this sieve. They were washed with F.A. fixative into a glass tube and stored. It was found to be necessary to mill before fixation, since the Waring Blendor cuts the fixed nematodes. Living nematodes were not injured. In the following weeks the contents of the tubes was diluted to 250 or 500 cc water, depending on the number of larvae present. The suspension was stirred and samples of 2,5 cc were taken with a Mac Donald pipette. Larval numbers were determined in counting chambers (Pl. XI, fig. 1). Six samples were counted from each root-system. The eelworms were now clearly visible between the root particles. Three larval stages (Pl. XI, fig. 2) could be distinguished: 1) larvae; 2) flask-shaped semi-adults and 3) males and females which were counted separately. Close attention should be paid to the sampling method. Since the weight of the root was known, the number of nematodes per gram of root could be calculated. Staining the suspension on the sieve with lactophenol-cotton blue was tried. However, the larvae which were soon to moult, did not absorb the stain.

RESULTS IN 1953

Some small experiments were made in 1953 and gave preliminary results which will be mentioned here. Three plots of pot plants were

1) This apparatus is very useful for nematological research on plant tissues (Taylor & Loegering, 1953). It was used in medical research ten years ago by Murray Fallis (1943).

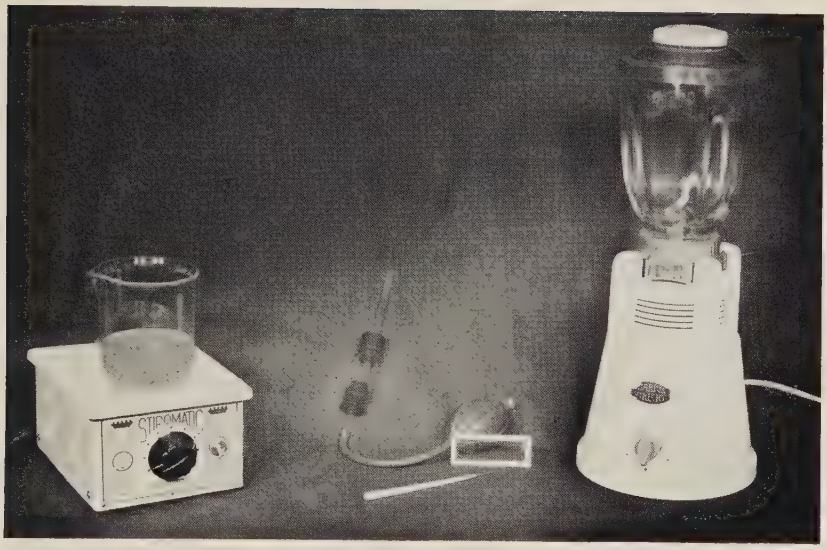

Fig. 1

Fig. 2

planted in soil to which fertilizer or farmyard manure had been added as well as in a none- manured control.

Every 10 days one or two pot plants was examined. Due to the great variability between plants the recorded differences were not significant. We determined the surface of the root-system of 10 days old plants and the nematodes it contained. The fertilized plants contained 105 larvae per square cm of root, the farmyard manured plants 40 and the none-manured control 64. Eight days later we found 3000 larvae per gram of root (fertilizer), 1088 (farmyard manure) and 1319 (none manured). Hence the number of eelworms present in plants growing in fertilized soil seems to be extremely high.

In one of the experiments a failure was noted which is worth mentioning: During a very hot weekend the 20 days old potplants were not sprayed and the glass house not aired. Shortly afterwards many plants died. However, the number of dead plants varied largely in the different series. Mainly infested plants growing in fertilized soil died, no doubt due to their already weakened condition (table II).

TABLE II

Experiment II 1953. Number of pot plants.

	Infested with cysts		Not infested	
	Dead	Alive	Dead	Alive
Artificial fertilizer	15	3	0	4
Farmyard manure	2	16	0	4
None manured	1	17	0	4

In other series, differences were also observed in the increase of cyst numbers at the end of the growing period. This cyst number increase was determined on two plants of each series (table III). The low rate of increase is due to the fact that this experiment could not be started before the middle of June.

TABLE III

Experiment I 1953. Number of cysts per 100 gram.

	Artificial fertilizer		Farmyard manure		None-manured	
	I	II	I	II	I	II
After	582	950	328	268	478	408
Before	210	210	210	210	210	210
Increase	372	740	118	58	268	198

As early as 1953, indications were found, that the influence of manuring on the severety of *Heterodera rostochiensis* infestation may be of importance.

RESULTS OBTAINED DURING 1954

One of the experiments was started on the 15th of April 1954. For this experiment sandy soil from a diseased field was used containing 189 cysts per 100 gr of soil. Four series, A, F, C, and N with 40 pots each were used.

A: soil + artificial fertilizer
F: soil + farmyard manure
C: soil + compost + artificial fertilizer
N: none manured soil only.

One potato-tuber of the variety Eigenheimer was planted in each pot. The pots were weighted after planting. Daily weight losses were made up by adding the appropriate amount of water lost. Six plants of each series were harvested at four different dates, namely, 12, 22, 33 and 78 days after planting.

Large differences were found between individual plants. It was therefore impossible to use data from 12 days old plants, since the larval penetration per root-system varied as much as 187 to 12.333 (table IV).

TABLE IV

Count I 1954. Plants nr. 1-6, 12 days old. Number of larvae per gram of root, mean of 6 counts.

Potplant nr.	A	F	C	N
1	119	59	155	417
2	133	370	148	2434
3	609	2297	77	760
4	443	1201	375	351
5	347	479	76	890
6	325	342	209	1990
Mean root weight:	6.5 g	6.1 g	5.9 g	5.7 g

(A = Artif. fert.; F = Farmyard man.; C = Compost; N = none manure.)

Several factors seem to be involved in causing this large variability. Though utmost care was taken when mixing the soil, it is more than likely that the cysts are not equally spread. The initial infestation of the plants would therefore not be identical. Furthermore, the reaction of the host is to some extent individual. Roots are dying and new roots formed and no plant reacts in precisely the same manner as another.

Far less variability was noted in 22 days old plants. Semi-developed larvae were now present in large numbers. The lowest total number of penetrating larvae per gram of root was found in the compost series; it was also low in the farmyard series but very high in the fertilized series. Moreover, the development rate of the nematodes in the various series showed differences. The percentage of semi-developed larvae (stage II) was distinctly lower in both the series, which had not received organic manure. All differences were statistically significant.

Especially remarkable were the low numbers of the second stage larvae in the compost series (table V).

TABLE V

Count II 1954. Plants nr. 7-12, 22 days old. Number of eelworms and percentage of different larval stages per gram of root.

	A		F		C		N	
	number	%	number	%	number	%	number	%
Stage I	1624	71	1027	86	627	95	878	86
Stage II	670	29	150	14	35	5	149	14
Total	2295	100	1186	100	662	100	1027	100
Mean root weight	15,4 g		17,7 g		17,9 g		15,7 g	

When examining the third group of harvested plants (33 days after planting), both males and females were found. However, the differences between the separate series, though pointing to the same direction as previously, are definitely smaller and statistically no longer significant. Only the percentage of females and males (stage III) was significantly lower in the organic manured series (table VI).

TABLE VI

Count III. 1954. Plants nr. 13-18, 33 days old. Number of eelworms and percentage of larval stages per gram of root.

	A		F		C		N	
	number	%	number	%	number	%	number	%
Stage I	397	29	364	38	286	36	298	29
Stage II	630	46½	427	45	369	47	406	39
Stage III (♂ & ♀)	323	24½	165	17	139	17	332	32
Total	1350	100	956	100	794	100	1036	100
Mean root weight	21.1 g		25.7 g		27.5 g		27.9 g	

This situation becomes more obvious when we consider weight results of the root-systems. Here we find that the fertilized series had the lowest weights, and the largest number of penetrated larvae. We therefore have a correlation between larvae contained in the root system and its weight. Especially, larvae in stage I and II were found to be starving. This seemed to be due to the fact that they attached themselves to the small rootlets. In the compost series, where the initial number of larval penetration in the root was small we now see high root weights. Thus a levelling of the population occurred, since numerous larvae died in the initiably severe infested plants and only relatively few in these moderately infested.

It is also of interest to consider the sex ratio of females and males. Apparently the sex ratio is distinctly higher than 1.0 and this, according to Ellenby (1954) points to a high infestation rate (table VII).

TABLE VII

Count III, May 18 1954. Number of stage III.

	A		F		C		N	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
number	683	603	453	256	413	301	1012	619
sex-ratio	1.13	1	1.77	1	1.37	1	1.65	1

The fourth count, (78 days old plants) met with difficulties, as by now white females had in part escaped and were free in the soil. The author's methods were not accurate enough to determine this number of females. Only the weight of the root-systems supplied interesting figures (table VIII).

TABLE VIII

1954. Count IV. Plants nr. 19-24, 78 days old.

	A	F	C	N
Root weights.	23.9 g	23.7 g	34.5 g	16.6 g

The differences between the series are now very large and highly significant. It was found that the largest root-system was developed in the compost plants. As the plants were full grown at this time, it was presumed that these root weights could be a standard for the ultimate final increase of the number of cysts in the soil.

The fifth count was made in October. Numbers of cysts per pot were determined. At the beginning of the experiment 5100 cysts per pot were found. Differences in count five were highly significant. The

largest number of cysts was formed in the compost-series. This result was unexpected, since the number of penetrated larvae in this series was always lowest. However, root mortality was also very low. Formation of new roots is always strong after compost manuring and hence the penetration of larvae in older plants results in a large number of cysts. OOSTENBRINK came to similar conclusions from his "long-year field plots" observations. Farmyard manure caused only a small increase of cyst population as was expected. If cyst numbers are divided by the root weights determined in July, a different picture results. We therefore calculated the number of cysts that would have been formed per gram of root, if the plants of counts four and five (harvested July and October) are presumed to be comparable. The number of cysts per gram of root is now equally low in both organic manure series (table IX).

TABLE IX

1954. Count V. Plants nr. 25-30 Number of cysts per pot in October

	A	F	C	N
Number (Oct.)	44390	34560	48610	31160
Initial number	5100	5100	5100	5100
Increase	39300	29400	43500	26000
Increase per gram of root	1637	1242	1261	1507

Farmyard and compost manuring both hamper the development of the larvae in the root. However, this retardation is slight and nevertheless many larvae reach their adult stage. This number is largest in the compost series due to the extensive root development towards the end of the season.

All experimental results of 1954 are summarized in a diagram (fig. 1), where a correlation of treatments may be noted.

DISCUSSION OF RESULTS

Organic manure in the form of farmyard manure or compost initially delays the development of the larvae of *Heterodera rostochiensis* on potato roots. Increase of the number of cysts in the soil is smallest in the farmyard series, larger in the fertilized series and largest in the compost series. This is due to strong root development which causes the development of large numbers of females towards the end of the growing period. The question which now arises is how to account for the arrest in nematode development in soils having received organic manure. There are several possibilities:

1) Organic manuring results in a better soil structure, as soil so treated has a higher water holding capacity. Because of this, many parasites cause less damage to their hosts in improved soil.

2) The number of micro-organisms in the soil increases after organic manuring. It may be that many nematodes are killed by their natural enemies (see the experiments of LINFORD).

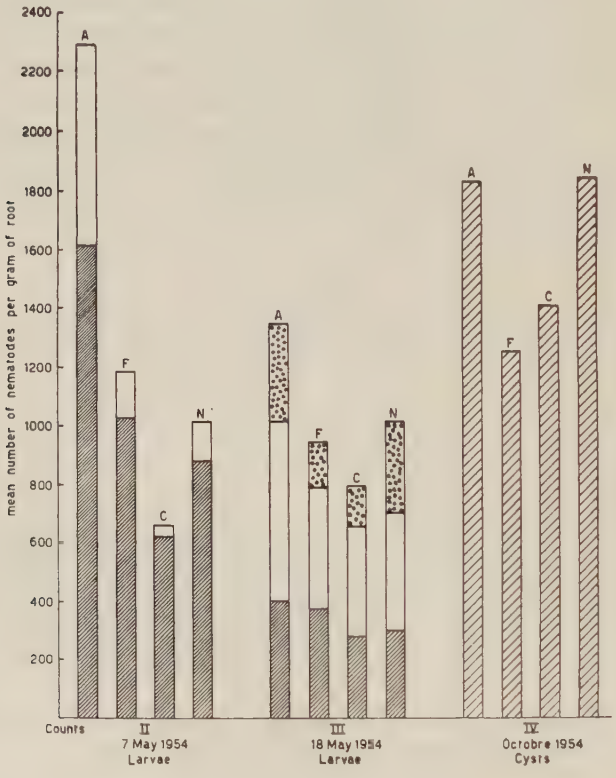

Fig. 1

3) Organic manuring affects the morphological structure of the plants, including the roots. Again it is possible that anatomic changes are responsible for the differences.

4) The changes caused by organic manuring in the plants, are also physiological. Each host replies to the penetration of a parasite with an antagonistic reaction. The varieties in plants in which this reaction is so strong that the parasite cannot reach its adult stage may be classed under a type of resistance. It may be suggested that a very light form of resistance is developed in the potato plants if organic manure is added to the soil.

In my experiments it was found that eelworms having penetrated *into* the roots of plants treated with organic matter, developed significantly more slowly than in plants treated with artificial fertilizer or in none-manured plants. No direct influence of soil conditions, such as structure (sub 1) or natural enemies (sub 2) can be the cause. It is however possible, that the anatomic changes (sub 3) hamper development. I myself, am more inclined to accept the reason given in sub 4. (physiological changes in the plant, resulting in a slight resistance), as the retardation is found not only in the beginning of the infestation when less larvae are found, but also later on, when the number of half grown and adult nematodes proved to be lower in the organic manured series than in those with fertilizer.

SUMMARY

Plant parasitic nematodes such as the potato root eelworm are obligate parasites. Investigations of such parasites are promising only, if both parasite and host are studied.

A method was developed to estimate the number of eelworms present in one gram of root.

Our experiments were confined to pot plants. Here it is possible at any required time to harvest the complete root-system. The roots were weighed, milled for 30 seconds in a Waring Blendor and stored in F. A. fixative. The suspension of root particles and eelworms was diluted in water and the different larval stages of the eelworms were counted.

In 22 days old plants significant numerical differences were found in eelworm population between the organic manure series (farmyard manure or compost) and the fertilizer series; those in the former being much lower. Moreover, the organic matter in the soil hampers the growth of the nematodes in the plants themselves. The percentage of the "second" and "third stage" larvae is lowest in the organic manured series. At the end of the growing period, however, extensive root production in the compost series caused the production of numerous cysts, but when calculated per gram of root the increase of cysts is largest in fertilized plants.

The influence of organic matter on the development of nematodes has been known for several years. Linford presumed that the increased development of their natural enemies in soil treated with organic matter might be the cause of this phenomenon. Hence, biological control of nematodes would take place when plants were treated with organic matter.

It was found in the author's experiments that eelworms, already penetrated into the roots of plants treated with organic matter, developed significantly more slowly than in those treated with fertilizer or in none-manured plants. No direct influence of soil conditions could be found to account for this.

It is concluded that plants treated with organic materials such as farmyard manure or compost, develop a slight resistance to nematodes. Physiological changes in the plant itself may be the reason.

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DISCUSSION

H. Goffart: 1) Wir haben ähnliche Versuche durchgeführt und können bestätigen das die Zystenbildung bei Kompost sehr hoch war; dann folgte in geringem Abstand Kunstdünger. Wir meinen, dass vielleicht auch die Bodenstruktur eine Rolle spielt, da die Nematodenlarven sehr sauerstoffliebend sind.

2) Es ist richtig, dass im Kompost zunächst erheblich weniger Larven schlüpfen als in anderen Böden. Wir vermuten, das vielleicht die Larven hier über einen längeren Zeitraum schlüpfen.

In diesem Zusammenhang wird auf Beobachtungen hingewiesen, nach deren

Wurzeldiffusate, wenn sie Sandboden passiert haben, zum 100% Aktivierungsreiz haben, wenn sie dagegen Kompostboden passiert haben, nur noch einen 40-50%-igen Aktivierungsreiz haben.

J. H. SCHUURMANS STEKHOVEN: asked if the observed differences may be caused by the fact that the roots of the compost-plants were growing faster than those of the plants which had received artificial fertilizer. This, rather than the becoming resistant of the roots, appears to him to be the real cause.

P. A. VAN DER LAAN: I do not think so, as the development within the roots also is retardated in the plants, receiving organic manure.

H. R. WALLACE: Experiments on the emergence of larvae from cysts of the beet-eelworm in various soil types show that there is some correlation between the rate of emergence and the oxygen-consumption of the soil which in turn is associated with the amount of organic matter.

I suggest that one factor which may contribute to the observed phenomena, is oxygen-competition between the nematodes and soil-micro-organisms.

I don't think the importance of manure and its effect on soilstructure should be underestimated, since crumb-formation and stability of crumbs does influence the rate of larval emergence in soil.

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THE UNKNOWN HATCHING AGENT FOR THE POTATO ROOT-EELWORM

(Some Purification Aspects)

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INTRODUCTION

It is well known that potato root-diffusate shows activity in hatching potato root-eelworm. A number of years were spent in purifying these diffusates, with the ultimate aim of isolating the hatching factor. Although some progress was made in purifying the crude diffusate, methods have not been satisfactory, owing to the many impurities to be eliminated from the crude preparation. Later, a simultaneous set of experiments were started from a different angle. MASSEY and NEAL showed, that an active substance is present in tomato leaves. This substance may be obtained by floating small disks from tomato leaves on distilled water. After one or two days the water shows hatching activity. The hatching factor seems to diffuse from the punches into the water. We repeated their experiments with potato leaves and also with other parts of the potato plant and were able to confirm their results. So far no hatching activity could be discovered in parts of potato tuber. The reason, we were interested in the active substance(s) of other parts from the potato plant is, that perhaps here lies a possibility of solving the problem of the hatching agent from a general viewpoint. A resemblance between these active substances seems possible.

In order to examine the active substance from leaves and stems we collected an amount of crude material by extracting the pulverized plants (except the tuber) with 50 p.c. alcohol. (This method is described in the second part). This way, a second raw "starting-material" was added to our first root-diffusate preparations. Other methods of purification were necessary to get information about the nature of the hatching agent. The methods used and the results obtained are described in the third part; the results are discussed in part four.

DESCRIPTION OF THE METHODS FOR COLLECTING CRUDE MATERIAL FROM ROOT-DIFFUSATE AND LEAVES (AND STEMS) FROM THE POTATO PLANT

1. *Collecting of the dry crude material from root-diffusate.*

Root-diffusate is filtered by passing a layer of charcoal. After two or three days the column is extracted with a mixture of benzene, acetone and water. After evaporating the organic solvents in a thin-layer-evaporator the residue is dried in an exsiccator at reduced pressure. The dry material is then stored in sealed tubes at 0-5°C. This is a modification of the method used by HURST and CALAM (1931-1939) and is therefore called the "classic" method.

2. *Collecting of the dry crude material from leaves and stems.*

The plants are ground (except the tubers) and mixed with 10 to 20 parts of approximately 50 percent alcohol. After 2 to 4 days the extract is filtrated. The alcohol is removed by evaporation. Chlorophyll can easily be removed by shaking with one third of volume ethylacetate. Although ethylacetate takes up some of the active material, the activity of the watery solution has been increased after chlorophyll removal. The ethylacetate still present in the water is evaporated and the watery solution is concentrated in a thin-layer-evaporator and afterwards dried in an exsiccator at reduced pressure. The dry material is stored in sealed tubes at 0-5°C.

DESCRIPTION OF PURIFICATION METHODS.

1. *Methods used with root-diffusate.*

a) Adsorption of the active substance on charcoal (norite) columns followed by elution with organic solvents, or organic solvents mixtures with water.

Result: Unsatisfactory, due to the poor adsorption of norite.

b) Extraction of the active substance from watery solutions by means of organic solvents slightly soluble or insoluble in water.

Result: Of several organic solvents, ethylacetate proved to be the best extracting liquid. On a bigger scale however, it is worthless because the solubility of the active agent in this solvent is still very poor.

2. *Methods of purification used with leaf-extract and root-diffusate.*

A. Removal of high molecular organic material and part of the inorganic substances (see fig. 1).

A concentrated solution of leaf-extract or root-diffusate in water is

mixed with 15 parts 96 p.c. methanol. The precipitate is filtered off and dried (N_1). A 10 p.c. solution of bariumacetate is added to the filtrate S (1) till pH 7.5 is reached. The formed precipitate is filtered off and extracted with a mixture of 96 p.c. alcohol and diluted H_2SO_4. $BaSO_4$ is removed by filtration and bariumhydroxide is added to the filtrate until pH 4.0. The newly formed $BaSO_4$ is filtered off again. The obtained clear solution is freed from methanol and dried (O_2).

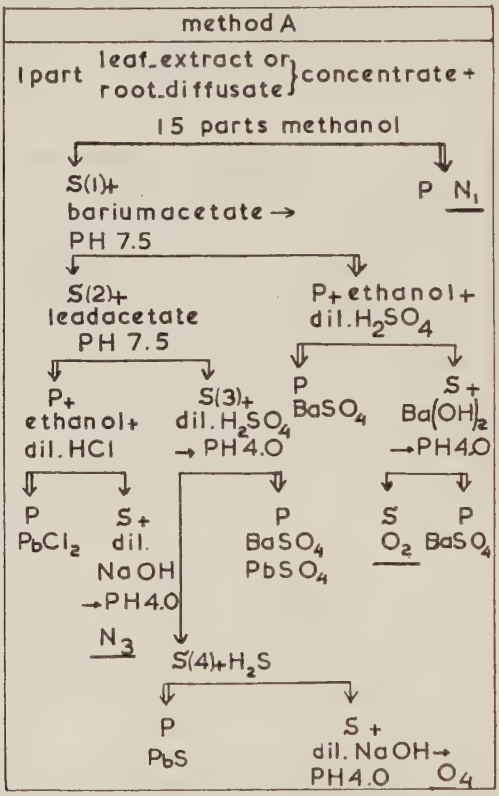

Fig. 1

The filtrate S (2) is treated with a 10 p.c. solution of leadacetate until precipitation ends. The pH is kept at 7.5. This precipitate is filtered off and extracted with a mixture of 96 p.c. alcohol and diluted HCl. $PbCl_2$ is filtered off and sodiumhydroxide added to the filtrate until a pH of 4.0. After filtration the solution is freed from methanol and dried (N_3).

On the other hand the filtrate S (3) is treated with diluted H_2SO_4

and filtered. The precipitate (Pb SO_4 and Ba SO_4) is discarded. The filtrate S (4) is treated with H_2S until all the lead has been precipitated. PbS is filtered off and the solution aerated in order to remove H_2S, and then freed from methanol. Sodiumhydroxide is added until pH 4.0. is reached. After filtration the solution is dried(O_4).

Parts of the dry fractions (N_1, O_2, N_3, and O_4) were diluted with water and used for the bioassay. Another part of the dry material was roughly examined on organic and inorganic material content.

The results of these experiments with leaf-extract and root-diffusate are described in 1 en 2 respectively.

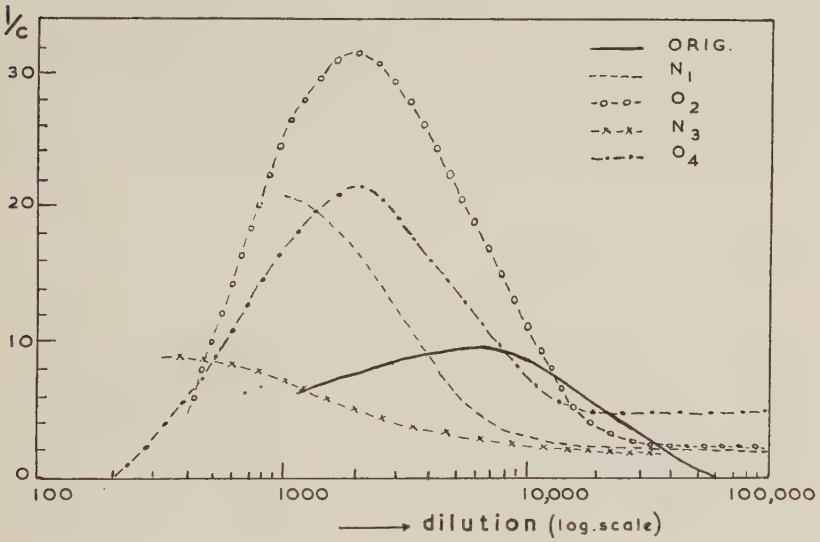

Fig. 2

1) *Results with leaf-extract by examining the purified fractions* (fig. 2 and table IA)

N_1: Yield about 25 p.c. of the original substance. The activity of

TABLE IA

Purification leaf- extract (Method A). Numbers of the experiment, dilutions and hatching numbers in larvae pro cyste

No.	Dil.	l/c	Dil.	l/c	Dil.	l/c	Dil.	l/c
Orig.	10^3	6	10^4	9	4×10^4	2	10^5	0
N_1	10^3	21	10^4	3	10^5	2	10^6	1
O_2	4×10^2	5	2×10^3	32	2×10^4	4	2×10^5	2
N_3	2.9×10^2	9	2.9×10^3	4	2.9×10^4	2	—	—
O_4	2×10^2	0	2×10^3	22	2×10^4	5	2×10^5	5

Water hatching 2 l/c.

N_1 seemed higher in comparison with the original in dilutions of about thousand times (fig. 2), but less active when more diluted.

The dry substance contained organic material (about eighty p.c.) and inorganic material (most of it consisted of potassium, calcium, some magnesium, oxalate, chloride and some sulfate). The ninhydrin reaction was positive.

O_2: Yield about 5 p.c. of the original substance. As a rule it was more active, consisting mainly of organic material with reducing properties. The ninhydrin reaction was positive.

N_3: Yield about 5 p.c., only slightly active. Ninhydrin reaction practically negative.

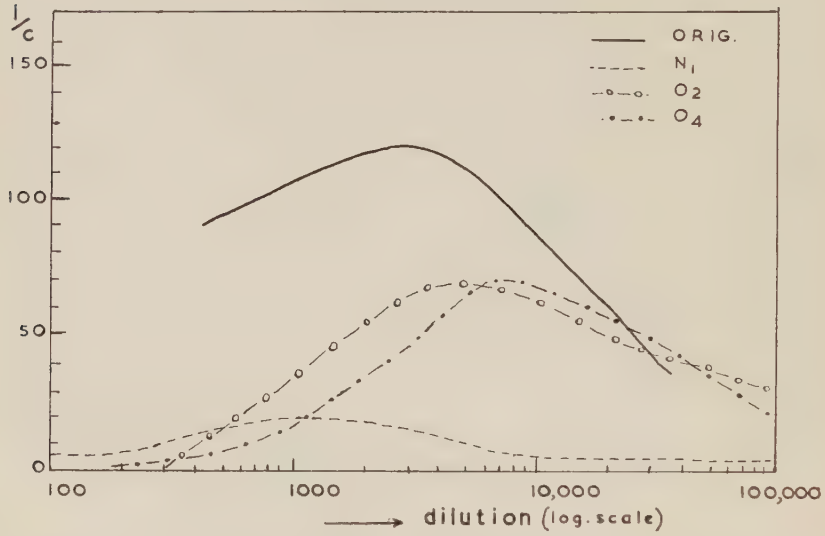

Fig. 3

O_4: Yield about 20 p.c., always more active than the original. Ninhydrin reaction positive; very hygroscopic organic material with reducing properties. Carbohydrates present.

2) *Results with root-diffusate by examining the purified fractions* (fig. 3 and table IIA)

N_1: Yield about 15-20 p.c. of the original substance, activity low, ninhydrin reaction slightly positive. Most of the inorganic part of the dry substance consisted of calciumcarbonate.

O_2: Yield about 5 p.c. of the original substance, activity reduced until about 50 p.c. of the original. Ninhydrin reaction negative.

TABLE IIa

Purification Root-diffusate (Method A). Numbers of the experiment, dilutions and hatching numers in larvae pro cyste.

No.	Dil.	l/c	Dil.	l/c	Dil.	l/c	Dil.	l/c
Orig.	4×10^2	90	4×10^3	121	4×10^4	37		
N_1	10^2	4	10^3	18	10^4	5	10^5	3
O_2	3.3×10^2	2	3.3×10^3	67	3.3×10^4	45	3.3×10^5	24
O_4	2×10^2	0	2×10^3	31	2×10^4	59	2×10^5	5
O_4	7.7×10^2	11	7.7×10^3	72	7.7×10^4	30		

Water hatching 2 l/c.

N_3: Yield about 5 p.c. of the original substance, activity very low, ninhydrin reaction negative.

O_4: Yield about 5 p.c. of the original substance, activity reduced till about 50 p.c. of the original, ninhydrin reaction slight positive; the O_4 substance was very hygroscopic, even more than the original substance.

B. Removal of inorganic and possibly organic material by means of ionexchangers.

This method was carried out with the following exchangers.

1) Amberlite I.R. 100, a strong Cation Exchanger (C.E.)

2) Amberlite I.R. 4 B, a weak Anion Exchanger (A.E.) and sometimes with: 3) Amberlite I.R. A 400, a strong Anion Exchanger (A.E.) We used small (30 ml) and bigger (165 ml) columns depending on the amount and concentration of the solutions from leafextract or root-diffusate. If we wanted to investigate the adsorption step by step we used the small columns.

After filling with the exchanger the columns were prepared for the adsorption stage in the usual way. Solutions of root-diffusate or leaf-extract in water were passed successively through the cation and anion column. In the eluates we measured the pH and the conductivity. A small part of each eluate was used for the bioassay. If necessary we repeated the adsorption procedure. For this purpose the columns were regenerated and the first anion eluate was passed successively through the cation and anion column etc. In these eluates the pH and the conductivity were measured again and small parts were taken for the biossay etc.

The pH of each cation fraction was brought to the pH of the respectively following anion eluate with sodiumhydroxide. The activities of the cation fractions are therefore not quite comparable with

the other fractions. However we thought it better to neutralize because, oherwise, the activity of the original solution could have been caused merely by the low pH. Sometimes we divided the cation eluate in two parts. The first part was passed through a weak anion column and the second through a strong anion column. So we could compare the two eluates especially on their activities.

In order to examine the adsorbed material on the columns we eluated them with diluted electrolyt after repeatedly washing with deionized water. As a rule we found that the eluates contained active material. This phenomenon is discussed in part four.

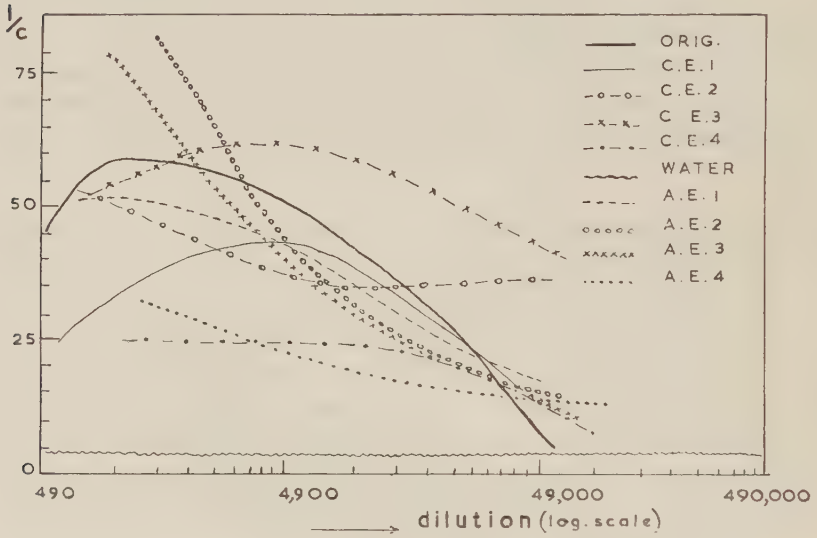

Fig. 4

The results of these experiments with leaf-extract and root-diffusate are described in 1 en 2 respectively.

1) *Results from examination of leaf-extract after passing through cation and anion columns* (see fig. 4 and table IB)

In this experiment we repeated the elution four times. We used the small columns and the weak anion exchanger. In figure 4 the activities from the second stage eluates are not quite comparable with the activities from the other eluates. We took in this stage cysts which were not presoaked as long as in the other steps. We further see that the activity from the third anion column-eluate is higher than the activity from the original extract (extract A), at least at a dilution of about

TABLE Ib

Purification Leaf-extract (extract A) (Method B). Numbers of the experiment, dilutions and hatching numbers in larvae pro cyste.

Number	pH	Spec. conductivity $\times 10^5$ (20° C)	Dil.	l/c	Dil.	l/c	Dil.	l/c
Extract A	5.9	—	735	55	7,350	42	73,500	4
„	5.9	566	490	39	4,900	52	49.000	22
1 × C.E.	1.6	1540	540	24	5,400	43	54,000	15
1 × A.E.	4.0	5.9	600	52	6,000	41	60,000	17
2 × C.E.	3.3	10.6	670	53	6,700	37	67,000	36
2 × A.E.	3.8	4.1	740	103	7.400	38	74,000	15
3 × C.E.	3.5	7.1	830	53	8,300	62	83,000	40
3 × A.E.	4.2	2.6	920	79	9,200	33	92,000	10
4 × C.E.	3.6	6.9	1,020	25	10,200	25	102,000	8
4 × A.E.	4.4	2.5	1,140	33	11,400	19	114,000	13

Water hatching 3 l/c.

500. The increase is reversed at higher dilutions. The fourth stage however is much lower than the original. In other experiments—not described here—we found the same phenomenon: in the first stages the activity *rises* and by repeating the experiment the activity *decreases*. Even the cation eluates, which are roughly on the same level as to activity as the original, show the same tendency. The pH's from the anion eluates remain about on the same level. The conductivity decreases gradually from stage one to stage four as could be expected.

In this experiment the anion columns were not eluated with diluted electrolyt.

2) *Results from examination of root-diffusate after passing through cation and anion columns* (see fig. 5 and table IIB)

In this experiment we used again the small columns and also a small column with strong anion exchanger. If we look at figure 5 we see that the activity from all the eluates were lower than the activity from the original. In other experiments—not described here—we found the same phenomenon.

As a rule we can say that the drop of the activity is proportional to the amount of exchanger or the dilution of the original solution.

It can be seen that the activity from the strong anion eluate is lower than the activity from the weak anion eluate. From other experiments we could confirm this result.

TABLE IIB

Purification Root-diffusate (Method B). Numbers of the experiment, dilutions and hatching numbers in larvae pro cyste.

number	pH	Spec. conductivity $\times 10^5$ (20° C)	Dil.	l/c	Dil.	l/c	Dil.	l/c	Dil.	l/c	Dil.	l/c
original solution	5.2	334	2×10^2	26	2×10^3	48	2×10^4	29	2×10^5	17	2×10^6	6
1 × C.E.	1.7	1100	2×10^2	56	2×10^3	26	2×10^4	28	2×10^5	12	2×10^6	11
1 × A.E (weak)	6.7	1.5	2.2×10^2	15	2.2×10^3	20	2.2×10^4	7	2.2×10^5	3	2.2×10^6	7
1 × A.E (strong)	7.0	1.7	2.2×10^2	3	2.2×10^3	4	2.2×10^4	6	2.2×10^5	9	2.2×10^6	6
HCL eluate A.E. weak	6.0	4.9	a	>42	10 × a	30						
HCL eluate A.E. strong	6.0	4.4	b	21	10 × b	15						

Water hatching 3 l/c.

After passing the solutions the columns were washed repeatedly with water and then eluated with 0.01 n HCl. A part of these eluates was used for the bioassay. The activity from the weak anion column-eluate (dilution unknown and therefore called "a") is practically on the same level as the original activity; the activity from the strong anion column-eluate (dilution unknown and therefore called "b") was much lower than the original (about 40 p.c.) In both eluates we could not detect any chloride.

In the following part the results of the two experiments—methods A and B—are discussed.

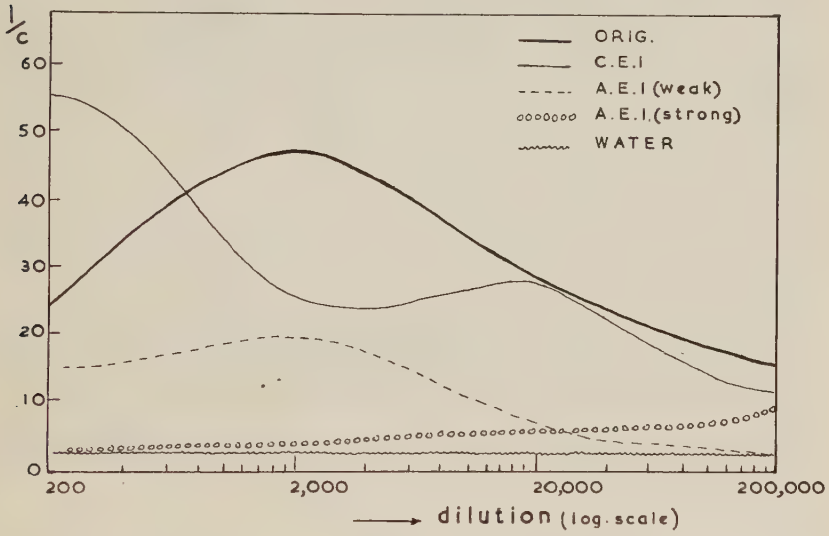

Fig. 5

DISCUSSION

Discussion of method A (see figs. 1, 2 and 3 and tables IA and IIA): By following this method it seems that we cannot get a good separation between active and inactive material.

By discarding inactive material, for example N_1, we also loose active material. Probably this is due to adsorption of active material on the precipitate. This purification method seems therefore not satisfactory. With regard to leaf-extract we can get more active fractions, (for example O_2 and O_4) but on the other hand we found in other experiments that removal of the first methanol precipitate (N_1) already gives a solution that is much more active than the original.

We therefore come to the following conclusion:

This method for the purification of root-diffusate is unsatisfactory. With regard to leaf-extract we prefer to stop at the stage where the methanol precipitate has been removed.

Discussion of method B (see figs. 4 and 5 tables IB and IIB):

With regard to leaf-extract we can get more active solutions if a certain amount of substance is adsorbed by the columns with exchangers. Perhaps this adsorbed material is an inhibiting substance. Besides, in the end we also loose active material (on the anion column). With regard to root-diffusate we loose active substance (on the anion columns) right in the beginning. If we use a strong anion column the drop in activity is greater. Adsorbed active substance can be eluated from the anion columns by using diluted electrolyte. (In this experiment we took diluted HCl). It seems that the HCl eluate from the strong anion column is less active than that from the weak anion column. We can not as yet explain this effect.

We therefore come to the following conclusion:

The active substance or a part of it is probably of an acidic nature. It seems therefore interesting to collect more of the adsorbed active substance on the anion columns. (On the other hand it is not yet clear which sort of anion exchanger should be preferred).

Until now we have no proof however that the active substance from the leaves and from the root-diffusate should be identical.

Much of the work dealt with in this publication was initiated by J. C. DE MAN, who left our Institute a year ago.

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DISCUSSION

H. DEN OUDEN: If in a clear solution of root diffusate at 2° C a precipitate is formed, could it be that this precipitate possibly has a stimulating activity?

G. J. JANZEN: It is not impossible that the precipitate, perhaps mainly consisting of organic impurities, shows hatching properties. This may be due to the fact, that some active material can be easily adsorbed by any precipitate.

M. OOSTENBRINK: If I am right you could calculate from your preliminary trials, that the amount of hatching agent in the leaves of a potato-crop is high enough to soak the whole tilth of that field 10 times with an active solution. Do you still think that the hatching capacity of the leaves is that high?

G. J. JANZEN: The calculating of Dr. OOSTENBRINK arises from an information that we could obtain a rather active solution by diluting 15 mgm of a dry leaf-extract to 1.5 L. These 15 mgm originated from one gram fresh leaves. On the

assumption that from one fullgrown potato plant can be gathered about 500 gm. of leaves, Dr. OOSTENBRINK comes to "the factor: 10 times". It was found in other experiments that this factor lies approximately between 2 and 20 depending on:

a) The potato variety
b) The growth of the plant
c) Perhaps the treatment of the soil.

Starting with dry leaves the factor lies approximately between 0,2 and 2, also depending on a, b, and c.

T. D. WILLIAMS: If you had tested the anion eluates (see fig. 4) more concentrated, would you have obtained the same shape of curve like that from the original?

G. J. JANZEN: As we have until now no disposal of a suitable "concentrating apparatus" (at low temperature) the concentrating action means loss of activity (originating from too high a temperature) and for this reason only causes a decrease in numbers of hatching larvae.

The shape of the curve, completed with the hatching numbers from a concentrate, could therefore not be compared with the curve from the original.

D. W. FENWICK: Is it possible to obtain a total hatch with the hatching agent from potato leaves?

G. J. JANZEN: In our experiments we never obtain the total hatching. We count the larvae after three successively hatching periods, each of four days. The thus obtained hatching numbers from root-diffusate ly between 50 and 200 larvae per cyst depending on

a) The age of the cysts
b) The size of the cysts
c) The seasonal variation in emergence.

For potato leaves these hatching numbers ly between 20 and 50 larvae per cyst.

In this way the larval emergence with leaf-extract never rises above 50% of that obtained with root-diffusate.

(*Nematologica*, Vol. I, 1956, no. 2)

THE INFLUENCE OF HOSTS AND NON-SUSCEPTIBLE HATCHING PLANTS ON POPULATIONS OF HETERODERA SCHACHTII

BY

H. DEN OUDEN

(Bergen op Zoom, the Netherlands)

The beet eelworm-research-program, at the Instituut voor Rationele Suikerproductie at Bergen op Zoom pays special attention to the control of beet eelworm by means of synthetic and plant-produced hatching agents. The aim of this work is to find a method that causes a more rapid decline of eelworm populations than that of the common crop rotation system.

In 1951 host-research was restarted in connection with JONES' work at Cambridge and HIJNER's at Bergen op Zoom, and aimed especially at the investigation of non-susceptible hatching plants. In 1950 HIJNER had shown the stimulating action within the genus *Beta* of the immune species *Beta patellaris* and we decided to limit our work, at least at start to the three most important host-plant-families Cruciferae, Chenopodiaceae and Polygonaceae. Those species of our indigenous flora which had as yet not been examined by JONES, received our special attention. Besides, we also wanted to determine the degree of reproductivity of a number of important Dutch weeds.

This latter experiment was made in the open in concrete microplots with representative soiltype for Dutch conditions.

The following species produced a population-increase of 200% or more in samples taken at 15 cm depth: *Alyssum maritimum, Stellaria media, Rumex maritimus, Sinapis arvensis, Teesdalia nudicaulis* and *Thlaspi arvense*. A 100-200% increase was given by *Atriplex hastatum, Capsella bursa pastoris, Chenopodium ficifolium* and *Nasturtium officinale*, whereas *Chenopodium album, Chenopodium rubrum, Atriplex littorale, Cardamine pratensis, Diplotaxis crucoïdes, Malcolmia maritima* and *Sisymbrium officinale* showed less than a 100% increase in viable cyst content.

Some factors which should be considered with regard to this reproductivity and in connection with our field-infestation with beet eelworm are mentioned here:

1) The frequency of the hostplant.

2) The life-cycle of the plant species.Biennial hostplants can be more dangerous than short-living annual weeds.

3) The nature and size of the rootsystem, which depends also on environmental factors. A very coarse rootsystem is less favourable to the development of eelworm than a fine fibrous one. A deeply rooted plant is more important as a host than a superficially rooting species.

4) The fourth point of importance is, to which degree the plant is physiologically suitable to the development of the eelworm.

When considering these factors the difference in reaction of species examined with regard to population increase is understandable. We will spare you the detailed results of the general tests for susceptibility because the list would be too long and because much of the data has also been published by WINSLOW from Cambridge. I only want to mention those plants which were found to be negative in our tests because a choice had to be made from this group for the hatching-plant examinations.

Next to the species *Chenopodium quinoa, Coronopus squamatus, Matthiola annua* and *Fagopyrum esculentum* which were indicated as negative by JONES also, no cysts were formed in our pot-tests on the following species: *Cakile maritima, Hesperis matronalis, Echinopsilon hirsutus, Kochia scoparia, Salicornia herbacea, Salsola kali* and *Suaeda maritima.*

Choosing a hatchplant from this series we had to consider first of all its eventual suitability for use in agricultural practice.

The halophytic species as such cannot be used whereas *Kochia scoparia* and *Chenopodium quinoa* by their annuality and abundant seed production might give rise to a serious weedproblem. *Fagopyrum* did not show any stimulating activity. The genera *Coronopus* and *Matthiola* stimulate the larvae of *Heterodera schachtii,* as experiments at Cambridge and Bergen op Zoom have shown. The practical use of these species however seems to be doubtful.

The Cruciferous *Hesperis matronalis* which according to our vitro-experiments (table I) produces a very active root-excretion was tested in field and greenhouse experiments.

This plant does not make too high demands with regard to soil, germinates quickly and at a moderate temperature, moreover grows with a fine fibrous rootsystem and is biennial.

The last character is especially attractive to the farmer because in practice the plant will not become a dangerous weed in the first year

TABLE I

Hatching experiments in vitro with Hesperis root excretions (H.r.e.), Beet root excretions (B.r.e.) and water.

Object		Water			B.r.e.			H.r.e.		
Time		1	2	3	1	2	3	1	2	3
Exp. I	1 week	24	4	6	222	10	22	298	11	27
Exp. II	1 week	0	0	0	148	7	21	172	16	11
	2 week	23	5	5	379	11	35	815	35	23
	Total	23			527			987		
Exp. III	1 week	201	6	34	479	11	44	806	18	45
	2 week	108	9	12	124	13	10	251	21	12
	Total	309			603			1057		
Exp. IV	1 week	138	12	12	1138	23	51	464	17	29
	2 week	596	26	23	980	27	36	1337	37	36
	Total	734			2163			1801		
Exp. V	1 week	360	20	18	1105	21	53	434	16	26
	2 week	225	25	9	959	31	31	750	29	27
	3 week	132	21	6	442	28	16	364	24	15
	Total	717			2506			1548		

1. = number of larvae per 50 cysts
Column 2. = number of cysts out of which larvae hatched
3. = number of larvae per (partly) emptied cyst.

of growth whilst the stimulation of larvae is continued until the autumn.

In 1954 experiments on a somewhat larger scale were made, these are being continued and repeated in 1955.

There may be four possibilities for the culture of this hatching plant: 1) as a maincrop; 2) under a covercrop; 3) as a fallow crop sown in July-August, and 4) as a mixture with a foddercrop.

Of the first possibility we have to be sceptical, although it is true that it's biennial cycle and its strong frost resistance gives us the chance to treat this plant like colza.

The seed yield seems rather good, whilst the quality of the oil is curiously quite the same as that of linseed oil, with exception of its scent; great objections to its culture as a maincrop are the difficulty of the introduction of a new product on the agricultural market and difficulties with harvest.

The culture under covercrop has been tried in 1954 and 1955 under barley and wheat on nematode-infested fields. The first growth was good but in the 3-4 leave stage the plant withered and became very susceptible to infestation by fungus parasites.

Development without covercrop was very good. In flax, which is known to be a better covercrop than wheat and barley and also under peas the plants looked healthier. We fear however, that after harvest there will not be sufficiently good plants left to cover the field.

The growth is retarded under the covercrop and it might be just as profitable to sow after the harvest of early crops such as seedpotatoes, flax and peas; this method of culture is mentioned under point 3 but does not seem to give the rootsystem sufficient time to penetrate the topsoil to a great extent during the warm summerperiod and so to stimulate the larvae of a reasonable number of cysts.

Finally, we have the culture of *Hesperis* mixed with a foddercrop e.g. lucern. The growth has had a good start this spring but the prospects of practical application are none too good, since it is not economical for the Dutch farmer to sow lucern in the spring without a covercrop; it may be better to sow the mixture after the harvest of early crops.

Mixed culture with this leguminous crop seems to be profitable for the growth of *Hesperis*; perhaps this is due to nitrogen suppletion and good aeration of the soil which is also beneficial for the stimulation of larvae.

The leaves of *Hesperis* are not poisonous to cattle and contain a good percentage of protein.

A series of greenhouse- and field-experiments were made in 1953 and 1954 to show hatching action in the soil; some of these results will be presented below.

A hatching experiment in the greenhouse, in pots with homoge-

TABLE II

Result of hatching experiment in Greenhouse pots

OBJECT	Total number of treated cysts.	% empty cysts.	% light-moderately filled cysts.	% heavily filled cysts.	Pw = 1 × % light + 3 × % heavily filled cysts.	% Population decline.
1. Carduus benedictus	170	71.2	20.0	8.8	46.4	7.2
2. Control	358	74.0	23.4	2.6	31.2	37.6
3. Coronopus Ruellii	206	77.2	22.8	0.0	22.8	54.4
4. Hesperis matronalis	183	83.6	15.3	1.1	18.6	62.8
5. Matthiola annua	213	80.2	17.4	2.4	24.6	50.8
6. Ruta graveolens	225	76.4	17.8	5.8	35.2	29.6
7. Ratio at start		70	20	10	50	

neously infected soil gave after eight months the following differences between plant-species (6 replications) (table II).

The population decline due to the influence of *Hesperis* amounted to 62.8%, whilst the control gave a percentage of 37.6% only. Other greenhouse trials, amongst which was a comparison with *Beta patellaris,* gave similar results. In a field-trial, (Latin square) *Hesperis* was examined with other plants and two synthetic hatching agents found to be active in vitro.

The average content per cyst was determined both in spring and autumn with the results given in table III.

TABLE III

Results of a Latin-square hatching experiment in the field.

OBJECT	% Average population decline	M	Significance of differences H	T	P	C	B
Melilotus	59,2						
Hesperis	71,7	12,5 xx					
Trifolium subterr.	63,2	4,0	8,5				
P.C.P. 10^{-2}	54,3	4,9	17,4 xx	8,9 xx			
Chlorcamphene 10^{-2}	59,8	0,6	11,9 xx	3,4	5,5		
Barley	53,0	6,2	18,7 xx	10,2 xx	1,3	6,8 x	

Furthermore, this population decline was also determined in eight plots where *Hesperis* grew from May to October as in the earlier experiment.

The results were combined with those from the earlier experiment and are shown in Fig. 1. The height of the columns give the population decline per plot, determined from average cyst content. These were analyzed in this case by means of BIJLOO's homogenisation-method.

About 250 cysts were examined from each plot. The variation, as is usual in this type of experiment, is rather large. However, the differences are reliable with more than a 99 percent probability.

It was very difficult to get comparable numbers of cysts per unit of soil in the spring and autumn samples, even with 9 borings per square meter. Therefore, in the results presented, the average numbers of larvae and full eggs per cyst are given; this shows the degree of viability of the population in a field, where we know that large numbers of cysts are present in the soil.

We also have to take into account that under a closed (fodder) crop

the biological activity in the soil is greater than under a grain-crop and in a bare stubble. Cystwall-decay therefore, proceeds quicker in the

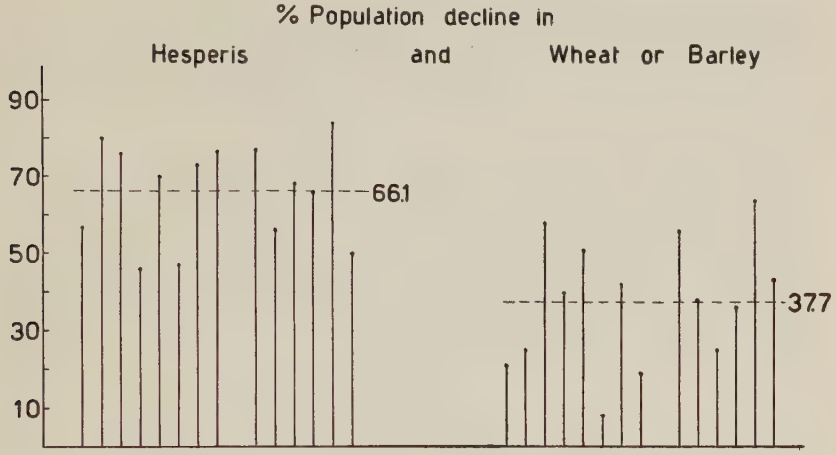

Fig. 1.

first case. The figures give therefore a relatively somewhat lowered impression of the real difference in population decline, which is explained in the fictive figures shown in table IV. From these it appears

TABLE IV

Example in numbers.

	Hatch crop		Control		Difference in Decline
	cy.: full cy.	% Decline	cy.: full cy.	% Decline	
Start in spring / Sample in autumn	100-100	—	100-100	—	
without cystwall-decay	100-40	60 %	100-70	30 %	30%
with more cystwall-decay in hatch crop	80-40	50 %	90-70	23 %	27 %
with equal cystwall-decay	90-40	56 %	90-70	23 %	33 %
with more cystwall-decay in control	90-40	56 %	80-70	12 %	44 %

that the measured difference in decline in our case is smaller than the difference both in the "trial" with the theoretical "no cystwall-decay" and in that with "equal cystwall-decay".

It is evident that cultivating a hatching plant even under the most favourable conditions will not result in complete disappearance of a beet eelworm-population. Cysts will always be left unreached by root-excretions and not all larvae will hatch within the first season. We can expect by these measures only, a shortening of the period during which the farmer cannot grow beets. It may thus be necessary to grow *Hesperis* for more than one year. This will be very difficult under normal Dutch croprotation, unless the mixed culture with lucern should be so favorable, that *Hesperis* will succeed as a perennial plant which can stay in the field two to four years.

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DISCUSSION

B. A. Cooper: Can I ask what is the effect of *Hesperis matronalis* on *Heterodera cruciferae*?

H. den Ouden: I have only tested the influence of plants on *H. schachtii*.

(*Nematologica*, Vol. I, 1956, no. 2)

THE EFFECT OF SOIL STRUCTURE ON THE EMERGENCE OF LARVAE FROM CYSTS OF THE BEET EELWORM

BY

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Reports from various sources during the last few years have suggested that the severity of eelworm attack and the level of the cyst population in the soil may be associated with soil type. Some of these reports have been contradictory and so the following observations based on laboratory experiments may be of interest.

Soil structure is a vague term but I define it here as the arrangement of the soil particles, the word particle meaning not only the individual elements such as sand, silt and clay, but also the units formed by an aggregation of these smaller fractions, commonly termed crumbs.

Observations on the relationship between soil structure and larval emergence entail, in effect, a study of the soil properties that depend on structure and their influence on the nematode. Thus the water and air relationships are primary functions of structure. These factors are, of course, only a part of a very complex environment, but by observing the eelworms under controlled conditions I think it is possible to assess the importance of soil structure The sintered - glass funnel technique was used throughout the experiments.

The results suggest that soil aeration is the most important single factor associated with soil structure which influences larval emergence. Pore-size, moisture content and air capacity in the soil are intimately related and their influence on larval emergence is really an expression of their effect on soil aeration.

The rate of larval emergence of the beet eelworm in coarse sand is high but there is a considerable decrease when small quantities of clay are mixed with the sand. At the optimum suction, about 3000 larvae emerged from 100 cysts in 7 days in the pure sand, about 1000 when the sand contained 1% clay, about 100 when there was 5% clay present and about 50 or less when 18% clay was present. The addition of clay reduces the size of the pore spaces in the sand and consequently the rate of diffusion of oxygen which in turn influences the rate of

larval emergence. This I think shows the importance of pore size. There is also a tendency for the rate of larval emergence to increase as the size of the soil crumbs increases. This, I think, supports the hypothesis that factors which tend to increase aeration favour high rates of larval emergence.

The effective pore space for air movement in a soil is influenced by soil moisture changes and consequently, soil aeration increases as the moisture content decreases. Measurements of the rate of larval emergence in different soil types have shown that as soil moisture is reduced, there is a corresponding increase in larval emergence until the water film is too thin for the larvae to emerge from the cyst. In soils which have a stable crumb structure this is clearly seen but in soils where the crumbs tend to break down on drying there is a uniform low level of emergence. These results are, I think to be interpreted as an effect of aeration. In soils with an unstable crumb structure the size of the pores is small and aeration is low, in soils which have stable crumbs the pores are relatively larger and aeration is consequently higher.

To sum up. Many of the factors which I have mentioned which influence aeration and larval emergence come under the heading of tilth. A soil is considered to have a good tilth when there is adequate aeration, sufficient moisture, ready infiltration of rainfall and so on. In fact, soil conditions which favour plant growth also favour larval emergence. The influence of aeration has been stressed but it should be pointed out that soil structure is only one factor which affects aeration. The oxygen consumption of the soil by the decomposition of organic substances and by microbiological activity has a big effect on aeration, and recent experiments with different soil types indicate that the rate of larval emergence may be determined by the oxygen availability in the soil I think that more emphasis should be laid on soil structure than on soil type, although it is probably justifiable to distinguish between light sandy soils and heavy clay soils. The consensus of opinion at the moment seems to support the view that more serious eelworm damage occurs on sandy soils. Work is at present in progress on the influence of soil structure on the migration of larvae through the soil.

I should be pleased to hear of anyone's experience of the relationship between soil type and eelworm damage.

(*Nematologica*, Vol. I, 1956, no. 2)

PREDATORY FUNGI AND THEIR UTILISATION IN NEMATODE CONTROL

BY

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The utilisation of natural enemies for the control of plant parasites has long attracted attention. If cheap and effective means of increasing the fungi, bacteria and viruses that attack plant parasites could be found, this method of control would be more rational than the employment of chemicals. Moreover, the biological means of control involves no toxic hazards to man as is often the case with chemicals. Especially promising is the use of micro-organisms which live as saprophytes in the soil but are capable, when there is an accumulation of harmful insects or nematodes, of resorting to the parasitic mode of life and of destroying some of the harmful organisms. Such are the so-called predatory fungi and among them is a group of soil fungi which destroy nematodes. These fungi develop special trap mechanisms by which the nematodes moving in the soil are caught. The first description of fungal traps was given by VORONIN (1869) and SOROKIN (1872, 1876). A more thorough study of predatory phenomena in fungi was made in France at the Pasteur Institute and in the U.S.A. in DRECHSLER's laboratory. In the U.S.S.R. the first comprehensive investigations of predatory fungi were initiated by F. F. SOPRUNOV (1947, 1950). Recently investigations have also been undertaken by the Moscow and Azerbaijan Plant Protection Stations.

Predatory fungi are widespread in the soils of the Soviet Union. They have been isolated from the soils of the extreme South and from the central zone of the U.S.S.R., e.g. Turkmenia and the Moscow region.

SOPRUNOV and GALIULINA (1951) have shown that the majority of predatory fungi found in the U.S.S.R. belong to the *Hyphomycetes*, are mainly distributed among the genera *Trichothecium* and *Arthrobotrys*, and to a lesser degree the genera *Dactylaria* and *Dactylella*. Certain forms of predatory fungi, isolated from the soil of Turkmenia,

have proved to be new species, viz. *Trichothecium pravicovi* (Sopr. & Gal.), *T. globosporum* (Sopr. & Gal.), *Arthrobotrys oviformis* (Sopr. & Gal.), *A. kirghizica* (Sopr. & Gal.).

Predatory fungi widespread in other places of the earth were also found, e.g. *Arthrobotrys oligospora* Fres., *A. longispora* Pr., *Dactylaria brochophaga* Dr., *Dactylella bembicoides* Dr. and others. Predatory fungi of the genera *Trichothecium* and *Arthrobotrys* have a morphological resemblance to the saprophytic species of the same genera, but differ from these in their ability to form ring-traps and in certain other physiological peculiarities. For instance, they decompose animal protein more rapidly than the saprophytes.

Experiments have been carried out on the application of predatory fungi for control of the root-knot nematode (*Meloidogyne marioni* Cornu), chiefly under indoor conditions, where the root-knot nematode causes damage in certain localities, mainly in cucumber and tomato cultivation. These experiments were first made by SOPRUNOV (1947) in Turkmenia. Predatory fungi were grown on plant media in Erlenmeyer flasks, maize and oat flakes or wheat bran being best suited for this purpose. Conidia of the fungus are sown on the medium and kept at 25-30° C, until the fungus grows through the entire thickness of the substratum. After that the contents of the flask are removed, dried and then ground into powder. If this powder is kept in a cold dry place the fungus retains its viability for not less than a year.

In the initial experiments, fifteen days prior to sowing cucumbers, ground medium containing the mycelium of predatory fungi was introduced into a flower pot with nematode-infected soil. Such powder usually contains large amounts of conidia. A count of galls on cucumber roots showed that, in the pots into which predatory fungi had been introduced, the number of galls was considerably lower. The controls averaged 23 galls/plant, while the pots into which predatory fungi had been introduced averaged 0.6 galls/plant. SOPRUNOV's experiments have shown that the greater the population density of nematode larvae in the soil, the more effective are the predatory fungi. The action of predatory fungi is reduced in conditions of excess moisture. Under favourable circumstances the predatory fungi remain active up to one month. SOPRUNOV has also demonstrated that the addition of ammonium carbonate heightens the effectiveness of predatory fungi.

In 1953 experiments were conducted by N. M. SVESHNIKOVA and by E. I. KONDAKOVA (unpublished) under the direction of the author

on soil containing root-knot nematodes. *Trichothecium pravicovi*, and *Arthrobotrys kirghizica* were used in the experiments; these were grown either on maize or wheat chaff.

Earthenware pots of approximately 4 litre capacity were used. They were filled with soil infected with the larvae of root-knot nematode, into which the fungi were introduced only once (first series), or four times (second series). In the first series the powdered culture was introduced in different doses: 0.1, 0.25, 1 % of the weight of the soil and thoroughly mixed with the total contents of the pots. Fourteen days later cucumber seeds were sown. In the second series, the fungus was introduced in the same manner but after that additional doses of the powder were introduced 3 times a week, the quantity being one fourth of the original dose. Thirty-four days later cucumbers were sown in these pots as well.

The final counts in these experiments showed that the infection in different treatments had decreased 1.5 to 7 times. For instance, in the case of a single introduction of the fungus the average infection of the material under test was 27.5 galls/plant. With doses of 0.25 % the result was an average of 12 galls/plant and with a dose of 1 %, about 9 galls/plant. In the second series 7 gr. to 4 kg. of soil, gave an average of 9 galls/plant, 17.5 gr. to 4 kg. of soil, approximately 5, and 70 gr. to 4 kg. soil, approximately 3 galls/plant respectively. The control of the second series showed an average of 18.6 galls/plant.

These experiments demonstrate the possibility of reducing root-knot nematode infection of plants by the use of predatory fungi. They also show that an increase in the dosage of fungus increases its effectivity. Finally, they demonstrate that the four-fold introduction of fungus produces a greater effect than a single introduction with an approximately equal amount of fungus. For instance, with a single introduction of 10 gr. powder to 4 kg. of soil, the infection of cucumbers with root-knot nematodes decreased 1.5 times, while with a four-fold introduction of 7 gr., infection was decreased 7 times.

Researches conducted at the Azerbaijan Plant Protection Station also showed that the introduction of predatory fungi into the soil reduces root-knot nematode plant infection.

Soprunov (1950) and Soprunov & Soprunova (1954) obtained positive results by somewhat similar methods in disinfecting the soil of certain nematodes pathogenic to man. They have also shown that fungi (*Didymozoophaga* spp.) develop predatory properties in alkaline and neutral media, whereas in acid environments this property fails

to manifest itself. The predatory habit appears to be a facultative property which may be enhanced by creating certain conditions in the soil. For example, the addition of ammonium carbonate heightens predatory activity.

Further development of the utilisation of predatory fungi in nematode control requires the creation of suitable soil conditions that will enhance predatory activity after introduction of fungi. Fungi isolated from different zones of the U.S.S.R. may require different soil conditions of temperature, humidity, etc. Therefore, to obtain the most satisfactory results in each zone, it will be best to utilize local species of predatory fungi.

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A REVIEW OF THE STUDY OF NEMATODES IN THE FAMILIES HETERODERIDAE AND TYLENCHIDAE, CAUSING CROP DISEASES IN THE U.S.S.R.

BY

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The study of plant parasitic nematodes has advanced considerably in the Soviet Union during the last two or three decades. Early studies were chiefly concerned with the fauna associated with cultivated plants and the rhizosphere, but free living nematodes have also been closely studied. These studies as well as the investigation of other problems in plant nematology are being continued. The development of methods of control as distinct from studies in morphology, biology and ecology, was stimulated by the discovery of parasitic nematodes causing considerable decreases in yield of cultivated plants.

Parasitic nematodes of the families *Heteroderidae* and *Tylenchidae* are being studied in different research institutes of the Soviet Union, the Academy of Science of the U.S.S.R., the Academies of Science of the Union Republics, the Lenin Academy of Agricultural Science and also in specialised plant protection institutes and stations, in industrial institutes and in colleges. The results of these studies are briefly reviewed in the present report.

THE ROOT KNOT NEMATODE

The root knot nematode, *Meloidogyne marioni* (Cornu), occurs in the U.S.S.R., having been found on vegetable crops grown in the open in the southern republics of the U.S.S.R., (Azerbaijan, Turkmenia, Uzbekistan, Armenia, Georgia, Ukraine). In some localities it does considerable harm. Several cases of attack by *Meloidogyne marioni* (Cornu) have been also recorded in more northerly regions (Byelorussian S.S.R., Lithuanian S.S.R., Latvian S.S.R., Moscow and Gorjkii regions of R.S.F.S.R. Attacks also occur under glasshouse culture.

In Russia it is considered that there is only one species of *Meloido*

gyne namely *M. marioni* (Cornu). Ustinov (1954) studied root knot nematode populations from different localities and came to this conclusion. However, he distinguished two forms; a southern form with large body size and a smaller northern form, possessing greater resistance to cold. Root knot nematodes are resistant to desiccation and survive soil conditions approximating to air dryness (about 1.8% moisture content). When crops are cultivated in such soil, root galling occurs, but only to a small extent.

The susceptibility to attack of some 300 varieties of crop plants has been tested and on this basis, plants have been placed in five categories (Shipinova & Ustinova 1954).

Group 1. Plants not susceptible to attack.

The roots are not invaded and grow normally, e.g. wheat grass, certain varieties of lucerne, sesame, safflower, peanut, *Vigna.*

Group 2. Resistant plants.

The roots develop small swellings or single galls containing larvae of the root knot nematode. Females do not reach maturity. The plants grow normally, e.g. many varieties of soya bean, the castor oil plant, *Vigna* 661 and a number of fodder grasses.

Group 3. Slightly susceptible plants.

Single galls are formed on the roots in which the females develop to maturity. Plants grow normally, e.g. sunflower, cowpea (varieties Brabham, Victor), certain varieties of lucerne, *Crotalaria,* millet, *Lallemantia,* maize, *Celosia cristata* L. (cock's-comb), Sudan grass, and Japanese millet.

Group 4. Moderately to highly susceptible plants.

The roots have numerous multiple galls. The roots decay and the plants remain stunted and eventually die. Most vegetable and melon crops belong to this group which also includes Haussan onion, vetch, beans, *Delphinium* sp. and many other cultivated plants.

Group 5. Highly susceptible plants which tolerate the presence of root knot nematodes.

Extensive galling occurs as in Group 4 but the roots do not decay and the plants grow normally, e.g. some varieties of hairy vetch,

peavine, Persian clover, buckwheat, linseed, cabbage, cauliflower, foxtail millet, cock's foot and some varieties of lucerne.

Vegetable crops belong mainly to group 3 and 4 with only a few falling in group 5.

Field experiments carried out by SHIPINOVA and USTINOVA (1954) have shown that although non-susceptible and resistant crops (e.g. barley and other cereals) reduce the nematode infection of the soil considerably (down to one larva/k.g. soil), even four years rest from susceptible crops is insufficient to rid the soil of root knot nematodes. For this reason the application of chemicals was developed in connection with crop rotation.

Experiments by SVESHNIKOVA and SHIPINOVA have shown that, of the various chemicals tested (cystogon, forbiat, preparations No. 23 and 47, petroleum oil waste, parathion, dichlorethane and others), the best results were obtained with forbiat (used as a standard) and preparation No. 23, the Soviet analogue. The application of 1-2 tons/hectare (0.4 to 0.8 tons/acre) of preparation No. 23 before the sowing or planting of vegetables, greatly reduced root infection. The number of healthy or slightly infected plants was 80% as against 100% badly infected roots in the controls. Rotting of roots was also eliminated. the plants growing and fruiting normally despite the presence of root infection. Preparation No. 23 also has a fungicidal and bactericidal effect on *Fusarium* and *Bacterium carotovorum* Jones. Tests of vegetables sown in treated soil made at the Department of Nutritional Hygiene in the Medical College, have shown that no toxic residue was absorbed by the foodstuffs. Because of the simplicity of application of preparation No. 23, it may be used to treat heavily infected patches in fields as an adjunct to crop rotation. It may also be used for vegetables grown under glass.

Steam sterilisation has also been developed as a control measure in greenhouses. Steam pipes of the 'comb' type are laid at a depth 30 cm. (12 in.) and steam is passed so as to raise the temperature to 100° C for three hours, the surface of the ground being covered with canvas or wooden shields (PARAMONOV 1951). Soil fumigation of glass houses with chloropicrin is also employed (up to 2 tons/hectare or 0.8 tons/acre), but neither steam sterilisation nor chloropicrin are completely effective and their application presents considerable technical difficulties.

The influence of low soil temperatures on root knot nematode survival in green house soil has been studied. Gall formation on roots

fails in soil frozen to — 3° C for three days; to — 7° C for not less than 2-3 days and to — 10° C for not less than 1 day (SVESHNIKOVA and PETROVA 1954). Further work is necessary before frost can be used in root knot nematode control.

Recently work has been started on the utilisation of predacious fungi for the control of root knot nematode and positive results have been obtained under growing conditions (GORLENKO, 1956).

THE POTATO ROOT EELWORM

The potato root eelworm, *Heterodera rostochiensis* Woll. was found in the U.S.S.R. in the autumn of 1940 on private plots in several towns of the Baltic Republics. No field infestations have been found here or elsewhere in the Soviet Union, despite annual surveys by State Plant Quarantine Inspectors. The study of this nematode began in 1949. It occurs in the soil to a depth of 40 cm., although most of the cysts lie between 10-20 cm. from the surface. Rates of soil infection from about 100 to 2,500 cysts/kg. soil are found. The attack upon potatoes occurs throughout the season regardless of planting dates. In the laboratory at 15-18° C, RASSINIJA (1954) observed infection of potato roots up to January. Therefore, in southern districts the development of more than one generation is possible. According to SVESHNIKOVA (1951), one and a half generations occur in the field under the conditions prevailing in the Baltic Republics.

Because the potato root eelworm attacks only certain members of the Solanaceae, the chief means of control is by crop rotation. Many chemicals, some in the form of dusts, have been tested for soil application but the best obtained so far was preparation No. 23. A single application to light sandy soil at 1 ton/hectare (0.4 ton/acre) reduced root infection by 83-93%; two applications at the same rate in autumn and spring gave 98% reduction. Even better results were obtained from two applications at the rate of 1.5 tons/hectare (0.6 ton/acre) namely, a drop of 20% in full cysts accompanied by a two fold increase in potato yield. In heavy soils the efficacy of the preparation is somewhat less. A 60% decrease in root infection was noted with a dosage of 1.5 tons/hectare (0.8 ton/acre). Potatoes may be planted on the same day as preparation No. 23 is applied. The compound may also be used in conjunction with crop rotation. Cysts are no longer found on the roots and the number of full cysts is diminished by 50% and that of empty cysts increased.

In the search for chemicals to eliminate potato root eelworm foci,

quarantine laboratories have experimented with the twotold introduction of dichloroethane in high doses (15-20 tons/hectare, 6-8 tons/acre), the soil being covered by mats for 15 days, but total elimination of the contents of cysts in the soil was not achieved.

Despite experiments with 30 varieties of potato no resistant types have been found.

THE BEET EELWORM

The presence of beet eelworm, *Heterodera schachtii* Schm., has been noted in certain old beet growing areas in the Ukraine. KORAB & BUTOVSKY (1939) and SCARBILOVICH (1954) have shown that this species attacks plants belonging to the *Chenopodiaceae* and *Cruciferae*. They have also worked out crop rotations including cereals and leguminous plants which reduce the nematode content of the soil. Chicory also reduces soil populations as also does the application of calcium hypochlorite to soil at the rate of 0.8-1.2 tons/hectare (0.32-0.48 tons/acre). Positive results have also been obtained with preparations of the forbiat type at 1 ton/hectare (0.4 ton/acre); Chloropicrin at 5-6 tons/hectare (2-2.4 tons/acre) failed to eradicate the beet eelworm completely (KORAB and BUTOVSKY 1939).

OTHER SPECIES OF HETERODERA

Isolated cases of the pea root eelworm [1]), *H. gottingiana* Lieb and the cereal root eelworm, *H. avenae* Filipjev, have been found as described by KIRJANOVA (1941). Hop root eelworm, *H. humuli* Filipjev, described by DMITRIEV (1954), occurs in several hop-gardens in the Ukraine where it is somewhat harmful to the plants.

POTATO TUBER EELWORM

The potato tuber eelworm, *Ditylenchus destructor* Thorne, has been found in the Ukraine, Georgia, Azerbaijan, particularly on the fields associated with distilleries. Experiments show that the tubers are the chief habitat of the nematodes and the main source of dissemination. Existence in the soil plays a minor part in the life cycle. The raising of healthy seed tubers and crop rotation are the only measures employed for the control of this nematode. Careful sorting of tubers before planting or storing is necessary. KIRJANOVA, LINNIK, BASSOVA,

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1) Named the alfalfa root eelworm in Russia.

Terestshenko, Ryss & Pogossjan (1954) found that summer planting, selection of healthy tubers for seed production, and lifting a fortnight early in the case of spring sown crops greatly reduced tuber infection. Experiments on the chemical disinfection of tubers have not given favourable results.

ONION STEM EELWORM

The onion stem eelworm, *Ditylenchus allii* Beijer., is found on onion plots, especially in private gardens and allotments where onion and garlic are planted year after year. Kirjanova (1951), Gerasimov (1954) and others have shown that onion seedlings become infected from residues of previous onion crops lying in the soil. The onion stem nematode attacks many varieties on onion and garlic but in tests could not be transferred to 32 genera of farm and garden crops. Small numbers have been noted on pumpkin, marrow, pea and sow-thistle (*Sonchus* sp.) but without the production of symptoms of disease. Kirjanova (1951) has observed decay of tomato stems infected with onion nematodes.

The principal source of onion nematode infection in new areas is onion seed. Control by crop rotation is recommended. It is also necessary to use clean seed. Onion seed and garlic transplants should be carefully winnowed before planting. The soaking of garlic and onion seed in water at 15-18° C for 3-4 days with change of water has been tried and produces 85-100% of healthy bulbs.

Dichloroethane at 1.5 tons/hectare (0.6 ton/acre) placed in the drills has also been tested for soil disinfection; 93% of the bulbs grown on such soil were healthy.

Preparations of the forbiat type had no effect on the onion stem nematodes.

PHLOX STEM NEMATODE

Phlox stem nematode, *Ditylenchus phloxidis* Kirjanova, has been found on flower growing farms. It has been observed on 127 varieties of phlox, inside the stem, leaves, sepals, and corolla. Leaf distortion arises from folding and the alternation of the sessile and peduncular habit (Sveshnikova 1946). Vassilevsky (1954) suggests the removal of diseased shoots accompanied by spraying with 0.04 % thiophos (0-0 diethyl O-P-nitro-phenylthiophosphate).

Further work is being undertaken by Soviet nematologists on the systematics, biology and ecology of plant parasitic nematodes and their

relationship with plant hosts. Cultural and chemical methods of control are being developed and the mode of action of nematicides and their effect on plants is also being investigated.

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DISCUSSION

F. H. JACOB: asked whether Dr. SVESHNIKOVA could disclose the composition of compounds No. 23 and 47.

N. M. SVESHNIKOVA: No. 47 is a rhodanine compound which works as a fungicide, but not as a nematicide. No. 23 is the ethyl ester of dimethyldithiocarbamic acid; its composition is mentioned in the published monographs which we brought and presented to the participants in the discussion. Forbiat is a compound which contains 20 per cent of dimethyl-dithio-carbamic methyl ester. Both No. 23 and forbiat have fungicidal action as well.

J. H. SCHUURMANS STEKHOVEN: Are the compounds No. 23 and 47 phytotoxic?

N. M. SVESHNIKOVA: They are not phytotoxic on some crops; but with other crops it is necessary to introduce preparation No. 23 into the soil 3-4 weeks prior to planting; the problem of phytotoxicity is still under study.

D. W. FENWICK: How was the 98 per cent reduction in root infection estimated?

N. M. SVESHNIKOVA: The number of infected plants on the test plots was compared with the number of those on the check plots.

M. OOSTENBRINK: How many "full time" nematologists are working in the U.S.S.R. to-day?

N. M. SVESHNIKOVA: 15 scientists of long standing and experience and many young scientists and agronomists also—about 50 persons in all.

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POPULATION STUDIES ON STEM EELWORMS (DITYLENCHUS DIPSACI)

BY

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The distribution of stem eelworms in the Netherlands shows a distinct relation to soil type. According to DEWEZ (1940) attack by stem eelworms in rye in the province of Limburg occurs only on the brown sandy river loam soils along the river Maas and on inland dune soil but this disease is of no importance on black sandy soils. If forest or heath is converted into arable land, stem eelworms spread very easily to these new fields if they are on brown sandy loam and dune soils but only very slowly or not at all if they are on black soils.

All Dutch river and marine clay soils seem to be infested with stem eelworms. However investigations on the island Goeree-Overflakkee by the Agricultural Advisory Service and the Dutch Onion Growers Association showed that onion bloat is a persistent menace on all heavy clay soils, whereas on light soils it only becomes important, when onions are grown too frequently i.e. more than once in three or four years. Fig. 1 shows two maps of the island Goeree-Overflakkee, one showing the areas which are suitable and non-suitable for growing onions, the other map showing soil areas containing over 30 % and below 30 % of clay particles. It is clear that there is a great degree of coincidence between the area non-suitable for onions because of the persistent menace of bloat and the area with clay content higher than 30 %. Also in other regions of the country stem eelworms occur on certain soil types and not on other types. Soil maps and geological maps show so much diversity in these regions that only a close comparison of the infested areas with detailed soil maps can confirm the impression which is obtained in the field.

Crop rotation practices cannot be the cause of the distribution of stem eelworms. On the contrary, the rotation follows the degree of eelworm infestation. Rye is grown more in uninfested and lightly infested areas in the sandy region than it is in heavily infested areas. Onions are grown on the very lightly infested light clay soils of Flakkee but are not common on heavy soils.

Fig. 1. Maps of Goeree-Overflakkee (scale 1 : 200.000) showing relation between soil type and occurrence of serious stem eelworm attacks in onions.

The degree of infestation of a susceptible crop, depends on two factors:

1. the degree of infestation of the soil at the time of sowing or planting;

2. The activity of the eelworms in the critical period. So the explanation of the influence of the soil on the occurrence of stem eelworm attack may be found in one or both of these factors. Data on the second factor are mentioned in an earlier publication (SEINHORST 1950).

The fluctuations in the degree of infestation of the soil with stem eelworms in relation to soil type and cropping practices are studied by investigation of soil samples. These samples are taken mostly in spring and in autumn on infested patches in fields of six infested regions: North Limburg, de Lijmers, Goeree-Overflakkee, West-Friesland, Western Noord Brabant and South Limburg. In each field, three to six areas of about 2 m^2 are sampled. The center of each of these sampling areas has been fixed by taking bearings on surrounding houses, light poles etc. and in this way they can be found again very rapidly and without measuring. On these 2 m^2 sampling areas 1 kg samples are taken, each composed of 60 subsamples (formerly 20 subsamples). In the laboratory the number of stem eelworms in 500 g of these samples is determined.

The life of the stem eelworms can be divided in two periods:

1. What might be called the active period: its life in a host plant.

2. A passive period: its life in the soil, after the infested plants have died or have been harvested.

Except when a susceptible winter crop is grown the winter is the most purely passive period, the summer more or less the active period. So, if we compare degrees of infestation in the autumn with those of the following spring we see in its purest possible form the decline of the number of stem eelworms in the soil by the influence of soil conditions. If however degrees of infestation in the spring are compared with those in the following autumn we see the influence of the crop and of the weeds. In table 1 a number of pairs of degrees of infestation in the autumn and the following spring are put together: in table 1 from heavy clay soils, in table 2 from light clay soils and sandy soils. It appears that the changes in numbers of stem eelworms in the heavy clay range from an increase of 1: 2.5 to a decrease of 6: 1 (mean 3.5: 1) where one component of the pair is over 100 stem eelworms in 500 g of soil. The increases

TABLE I

Decrease of the number of stem eelworms in different soils during winter

A. Heavy soils.

District:	West-Friesland				
Field:	Blank			Compas	
Autumn	1112*	144	33	177	112
Spring	366	29	18	66	48

District:	Goeree Overflakkee												
Field:	Vroeg in de wei			Tanis			Mastenbroek			Voogt	Jansen		
Autumn	60	142	58	**82**	56	**31**	**85**	**59**	**57**	**158**	**307**	1
Spring	46	93	10	173	31	46	19	47	77	72	77	21

District:	West-Brabant									
Field:	Bom				v. Dijken					
Autumn	**53**	**387**	**362**	**284**	**99**	**118**	**136**	**283**	**40**	**0**
Spring	137	163	104	79	13	39	21	109	45	5

* Numbers of stem eelworms in 500 g of soil. Bold type: degrees of infestation after onions.

B. Light soils.

District:	Goeree Overflakkee				W. Brabant		Ottersum					
Field:	Kastelein		Mol		v. Es		4/37					
Autumn	**564***	85	15	5	**382**	**825**	**347**	**98**	10	9	3
Spring	125	5	5	0	8	70	69	4	8	2	1

Field:	3/11 and 3/36			v. Bergen			3/04		Freriks			
Autumn	**1340**	109	16	**145**	**669**	**470**	29	3	9	4	17	2
Spring	147	48	3	34	197	50	35	4	1	0	0	4

District:	Vierlingsbeek-Maashees															
Field:	Nelissen			Welles I		Welles II				Bloemen			
Autumn	**313**	**8**	78	260	27	28	55	45	15	127	81	292	204
Spring	21	0	17	35	0	1	4	4	3	48	41	61	33

* Numbers of stem eelworms in 500 g of soil. Bold type: degrees of infestation after onions, rye or oats.

show the influence of sampling errors but they may also be partly due to multiplication on weeds, or passive or active migration of the eelworms from places with a higher degree of infestation.

On the lighter soils the decreases range from 2 : 1 to 45 : 1. Even if we exclude the numbers obtained on light clays (v. Es, Kastelein) the range is 2 : 1 to 15 : 1 (mean 6.7 : 1), which still does not appear to be very consistent.

Nevertheless we may conclude that on the sandy soils and light clay soils the stem eelworm population in the soil decreases more than on heavy clay soils and this tendency is still more apparent when we compare the decrease of autumn degrees of infestation of 20-60. On heavy clay soils nearly half of them show an increase, on the other soils even numbers of about 10 still show a general tendancy to decrease. It appears that on the heavy clays the degree of infestation shows a tendancy to stabilize at a level somewhere round 50 stem eelworms in 500 g of soil. Growing onions may bring up this level considerably while growing non susceptible crops does not reduce the number of stem eelworms in the soil below this level. On the lighter soils however, the degree of infestation decreases rapidly to levels which nearly escape our means of detection. Here onions, rye and oats are the only crops causing a high degree of infestation and we may conclude that all other susceptible and sometimes heavily infested crops like mangolds, potatoes and peas as well as non susceptible crops like wheat and barley cannot maintain considerable numbers of the stem eelworms in these light soils.

We suppose that both on the heavy and on the light soils weeds are responsible for the maintenance of minimum levels of infestations. The importance of weeds may be illustrated by the numbers of stem eelworms before and after (nonsusceptible) wheat on a field in South Limburg: 29 and 8 before sowing, and 140 and 55 resp. after harvesting the wheat. The main weeds here were the highly susceptible Stellaria media, Anagallis arvensis and a Galium species.

It may be seen from the tables, that the numbers of stem eelworms found in soil samples are rather low even after heavily infested crops. Generally there are less than a hundred and very often less than 50. Such numbers will always show a very high coefficient of variation. Even high numbers are not so much better in this respect because of a very irregular distribution of stem eelworms in the soil after infested rye, oats, or onions which are sown in rows, about 25 cm apart.

In table 2 we see that even 10 eelworms in 500 g of soil may cause very serious damage to onions. At about 25 eelworms in 500 g of soil there is no healthy plant left in the field.

TABLE 2

Relation between the number of stem eelworms in 500 g of soil and the degree of infestation of onions

Number of stem eelworms	Disease index	Number of stem eelworms	Disease index	Number of stem eelworms	Disease index	Number of stem eelworms	Disease index
6	6	16	8	28	7	0-10	7
7	8	19	4	29	1	10-20	5
9	8	20	1	32	1	20-30	3
10	4	25	4	36	2	> 30	2
14	7	26	3	43	3		
14	4	27	5	44	1		
14	2	27	2	79	2		

Disease index: 10 = no disease
1 = all plants bloated.

From a limited number of observations the impression was obtained that with rye the situation may be the same. Here, in some cases, 10-40 stem eelworms in 500 g of soil caused considerable damage. In the autumn after the crop, such a degree of infestation may have increased 20-100 fold.

So the differences shown in table 1 may certainly result in a persistent menace to onions on heavy soil, and a reasonable chance of a healthy crop on the light soils.

There is no indication as yet by what factors the mortality of stem eelworms in soil is influenced. They seem to be specific however. Heavy soils which appeared to be very suitable for stem eelworms often contain low numbers of other species of nematodes (total number of eelworms in 500 g of soil ± 5000 or lower) whereas sandy soils free from stem eelworms may contain total numbers of other eelworms of 10 000-30 000 in 500 g of soil.

In these soils, plant parasites like *Pratylenchus pratensis, Tylenchorhynchus* sp. and *Hoplolaimus uniformis* occur very commonly and often in high numbers. *Hoplolaimus uniformis* is rare in clay soils but *Pratylenchus thornii* seems to prefer them. So it is clear that in order to solve some of the problems round population fluctuations of plant parasitic eelworms, much attention will have to be paid to conditions in the soil as one of the major factors in their ecology.

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DAS CHAMPIGNON-MYZEL ALS INDIKATOR FÜR DIE WIRKUNG SAPROBER NEMATODEN IN KOMPOSTEN

VON

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Mit den organischen Düngern, z.B. Pferdemist- oder Strohkomposte, gelangen neben den Mikroorganismen eine Menge von Kleintieren in den Boden. Je nach dem Alter der Komposte können saprobe Nematoden, vor allem *Rhabditiden*, ausserordentlich stark vertreten sein. Ob ein Massenauftreten der Saprobionten unsere Kulturpflanzen zu schädigen vermag, ist bisher unbekannt. Gelegentliche Beobachtungen darüber, dass ausgetragener und stark mit *Rhabditiden* verseuchter Champignon-Kompost das Wachstum von Pflanzen nachteilig beeinflussen kann und Ertragsverminderungen in strohgedüngten Parzellen deuten jedoch eine Möglichkeit an.

Das Champignon-Myzel reagiert sehr empfindlich auf grössere *Rhabditis*-Mengen. Es lag daher nahe, zunächst das Verhalten der Tiere in den Champignon-Komposten und ihren Einfluss auf das Myzel zu studieren und dann erst die Vorgänge an den grünen Pflanzen. — Über den ersten Teil meiner Arbeit werde ich nun berichten.

A. RHABDITIS-ARTEN IN CHAMPIGNON-KULTUREN

In der Literatur wurde verschiedentlich über das Auftreten bestimmter Nematoden-Arten in Champignon-Kulturen berichtet: Cairns & Thomas (1950); Moreton (1953); Rempe (1954); Rempe & Kux (1953); Seinhorst & Bels (1951); Thomas & Mitchell (1950). Ich hatte Gelegenheit zahlreiche Kompostproben aus solchen Beeten zu untersuchen, die vorzeitig stark in ihren Erträgen zurückgegangen waren. Die Tabelle I gibt einen kleinen Ausschnitt davon wieder. Die Tiere gehörten vorwiegend der artenreichen Gattung *Rhabditis* an; am häufigsten waren vertreten: *Rh. cylindrica, curvicaudata, brevispina, elongata, longicaudata, producta* und *Rhabditis teres,* in geringen Mengen *Diplogaster-* und *Cephalobus*-Arten und *Tylenchiden* (s. S. 168).

Bei Anwesenheit von *Rhabditis*-Arten kann die Champignon-Ernte im Anfang der Kulturperiode noch gut sein. Früher oder später bil-

TABELLE I

Rhabditis-Arten in Deutschen Champignonkulturen. Fruchtkörperbildung stark zurückgegangen bzw. fehlte ganz.

Züchter	Nematoden/10 ccm Kompost I	II	III
A	430	785	1260
B	446	1184	3950
C	716	1228	1437
D	917	1034	6708
E	470	692	1965
F	565	1648	7084
G	493	643	1260
H	1443	3507	4378
I	873	1157	5619
J	525	621	4831
K	732	4831	8370

den sich aber auf der Deckerde freie Stellen, an denen die Bildung der Fruchtkörper ausfällt. Häufig stellen die noch kleinen Fruchtkörper ihr Wachstum ein und sterben schliesslich ab (Tafel XII). Bei sehr stark verseuchten Komposten wächst aber schon die Champignon-Brut nur zögernd an und die 1. Ernte fällt recht spärlich aus.

Die Ertragsverminderung, die durch *Rhabditis*-Arten verursacht werden kann, zeigt in ihrem typischen Verlauf die graphische Darstellung (Abb. 1): Der mit *Rhabditis* infizierte Kompost brachte nach 7 Erntewochen insgesamt nur 2,8 Kg Champignons/m^2; weitere Fruchtkörper wurden nicht mehr gebildet. Im Kontrollversuch waren die Tiere durch Erhitzen des Kompostes und der Deckerde auf 56-58° C abgetötet worden; nach 12 Wochen belief sich der Ertrag auf 6,82 Kg/m^2. (Die Kultur steht z.Zt. noch im Ertrag.) Die Gegenwart der Nematoden kann zu einer Alkalisierung des Substrates führen. Bei Überschreiten einer bestimmten Populationsdichte, nämlich 400 bis 700 Rhabditiden/10 cm^3 Kompost („kritischer Wert"), sinkt die Fruchtkörperbildung rasch ab (vergl. Abb. 1). Wenn der kritische Wert bei geringer Fruchtkörperbildung sehr stark unterschritten wird, so ist das in der Regel ein sicheres Zeichen für die gleichzeitige Wirkung anderer schädlicher Faktoren.

Zu welchem Zeitpunkt eine von *Rhabditis* befallene Kultur keine Erträge mehr bringt, hängt neben der Höhe der Ausgangspopulation wesentlich von der Feuchtigkeit und von der Temperatur ab: Im Experiment wurde in Komposten bei gleich hoher Ausgangspopulation bei niederer Feuchtigkeit und Temperatur (9-10° C) der „kritische Wert" nach 5 Monaten erreicht, bei höherer Feuchtigkeit und Tem-

peratur (14-16° C) dagegen schon nach 2½ Monaten stark überschritten (1400/10 cm³).

Bei längerer Benutzungsdauer der Kulturräume kann der Ertrag an Champignons abnehmen, eine Erscheinung, die als „Raummüdigkeit" bezeichnet wird. Allen derartigen Fällen war ein stärker Befall der Grundbeete mit *Rhabditis*-Arten gemeinsam. Wenn nach beendigter Ernte der alte Kompost entfernt wird, verbleiben viele Tiere, teils in Trockenstarre auf dem Boden der Kulturräume (oft natürlicher Boden) und dringen später in die neuangelegten Grund-

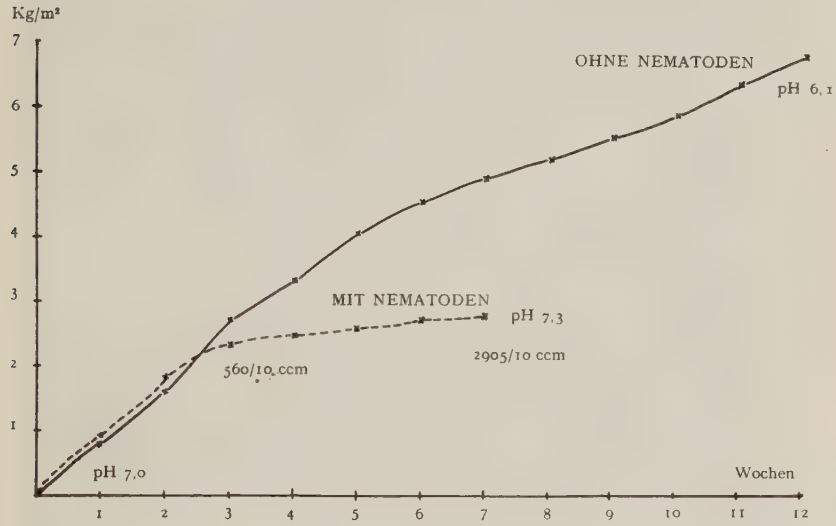


Abb. 1.

beete ein. Auch der Kompost, der auf den schon jahrelang benutzten Kompostplätzen präpariert wird, kann stärker als normal infiziert sein. Beim Übergang zur Pasteurisierung des Kompostes und der Deckerde und bei Verwendung von Kisten- oder Etagenbeeten, die ja nicht mehr direkt auf dem Boden stehen, verschwindet diese „Raummüdigkeit".

B. NEMATODEN IN DEN AUSGANGSMATERIALIEN DER KOMPOSTE UND DER DECKERDEN

Die Nematoden gelangen über die Komposte und die Deckerden in die Champignon-Beete.

K o m p o s t. Die Tabelle II zeigt, dass ausserordentlich hohe Mengen an *Rhabditis* in den Ausgangsmaterialien der Champignon-

Komposte (roher Pferdemist, Strohgarben, Ballenstroh, Sägemehl) enthalten sein können. Es handelt sich im wesentlichen um die bereits erwähnten *Rhabditis*-Arten. Die Untersuchung des Strohes wurde in

TABELLE II

Anzahl der Rhabditiden/10 ccm in den Ausgangsmaterialien der Champignon-Komposte

Material	Mittel aus 200 Einzelbestim.	Mindest bezw. Höchstwerte
Pferdemist, roh	6.990	731- 24.570
Strohgarben, trocken (Bauer A)	18	2- 67
Strohgarben, trocken (Bauer B)	92	8- 421
Ballenstroh, trocken (Bauer C)	283	27- 631
Ballenstroh, feucht (Bauer C)	16.350	3.250-135.460
Sägemehl, feucht	1.540	5- 9.470

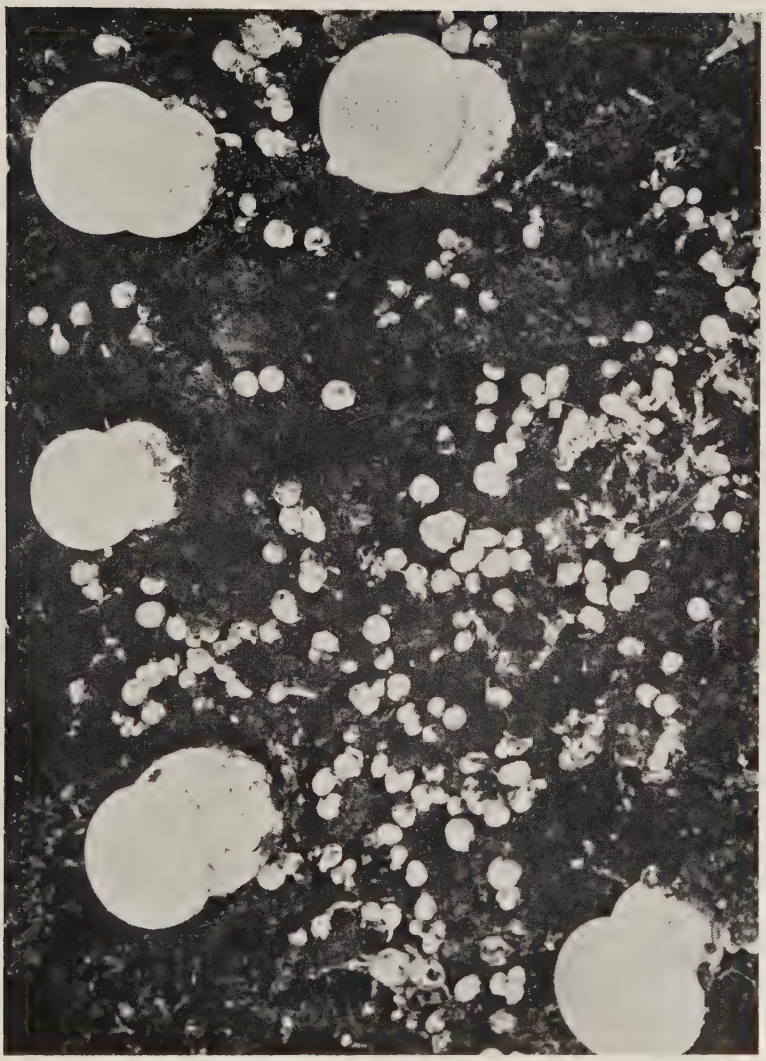
dem regenreichen Herbst 1954 gemacht. Die Äcker selbst wiesen eine hohe Zahl an *Rhabditiden* auf, weil der Bestandsabfall der Pflanzen durch die starken Niederschläge bereits im Herbst in eine grössere Zersetzung eingetreten war. Sehr stark waren Strohgarben verseucht, die längere Zeit auf den Äckern verblieben und besonders solche, die auf dem Boden lagen (Mähbinder). Recht zahlreich war übrigens *Rhabditis cylindrica* vertreten; die Tafel XIII zeigt eines der häufiger zu beobachtenden Kopulationsstadien.

Mit dem Eintrocknen des Strohes gehen die Tiere in die *Trockenstarre* über. Dabei können sich hunderte von Nematoden zu kompakten kugelförmigen Gebilden vereinigen. Die Tafel XIV zeigt ein derartiges kleineres Nematoden-„Aggregat" kurz nach seiner Anfeuchtung und in Wärmestarre. Die Tiere (*Rh. elongata*) sind mit ihren Schwanzenden verklebt. Die *Rhabditiden* gelangen mit dem Stroh in den Pferdestall.

Deckerde. Ausserdem finden die Nematoden über mangelhaft oder garnicht pasteurisierte Deckerden Eingang in die Kulturen, u.a. auch Tylenchiden, wie z.B. *Paratylenchus, Tylenchorhynchus, Rotylenchus, Aphelenchus* und *Aphelenchoides*.

C. DAS QUANTITATIVE VERHALTEN DER NEMATODEN IM VERLAUFE DER KURZKOMPOSTIERUNG

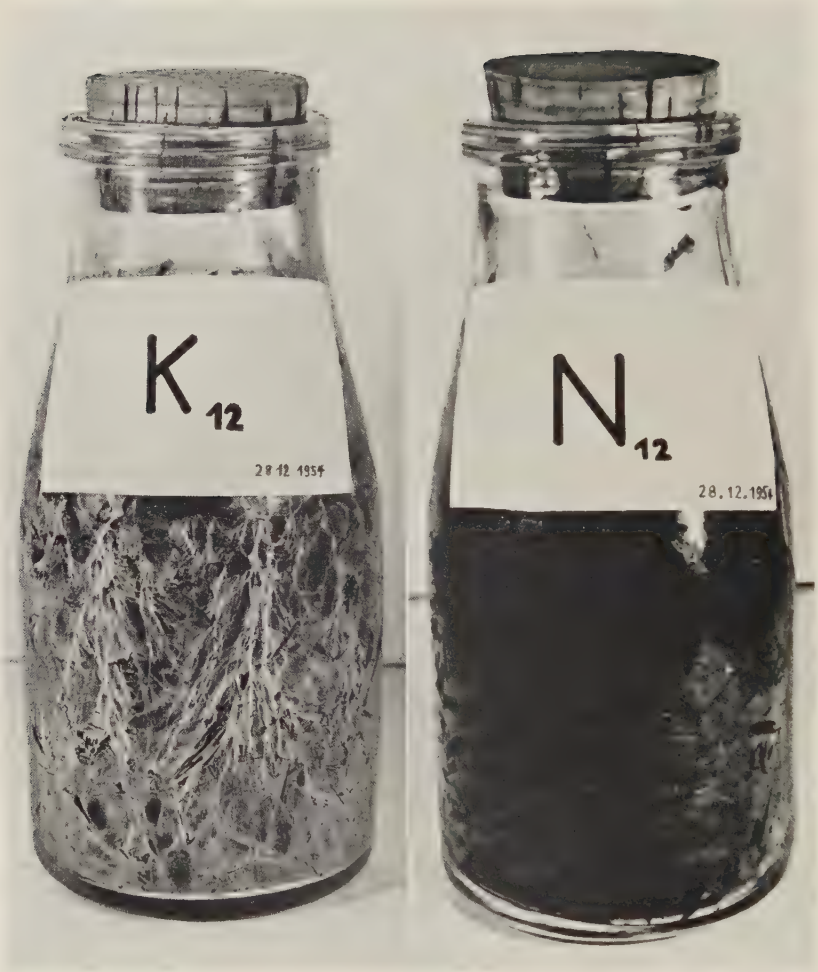
Der Nematodenbestand in den Ausgangsmaterialien der Champignonkomposte kann im Verlaufe der Kurzkompostierung stark reduziert werden. (Abb. 2): Ein frisch angesetzter Düngerhaufen enthielt z.B. im Durchschnitt 2400 *Rhabditiden*/10 cm³ (*Rh. cylindrica, elongata, teres, brevispina*). 3 Tage später, kurz vor dem 1. Umsatz



Massenauftreten von *Rhabditiden* in einem Champignon-Beet. Die kleinen Fruchtkörperchen stellten ihr Wachstum ein und starben ab. Die dunklen Faulstellen wurden secundär von den Tieren befallen.

Rhabditis cylindrica, Kopulationsstadium

Rhabditis elongata, kugelförmiges Nematoden-„Aggregat" kurz nach seiner Anfeuchtung. Die Tiere sind noch mit den Schwanzenden verklebt.



Flaschen-Kulturen, beimpft: (K) mit Bakterien, (N): mit *Rhabditis*-Arten und Bakterien.

des Haufens waren lebende Tiere nur noch in der etwa 25 cm dicken Aussenschale (21-40° C) vorhanden, während sie im Innern bei Temperaturen bis zu 72° C und mehr abgestorben waren. Die Populationsdichte in der äusseren Schale war aber etwas gestiegen (3250/10 ccm),

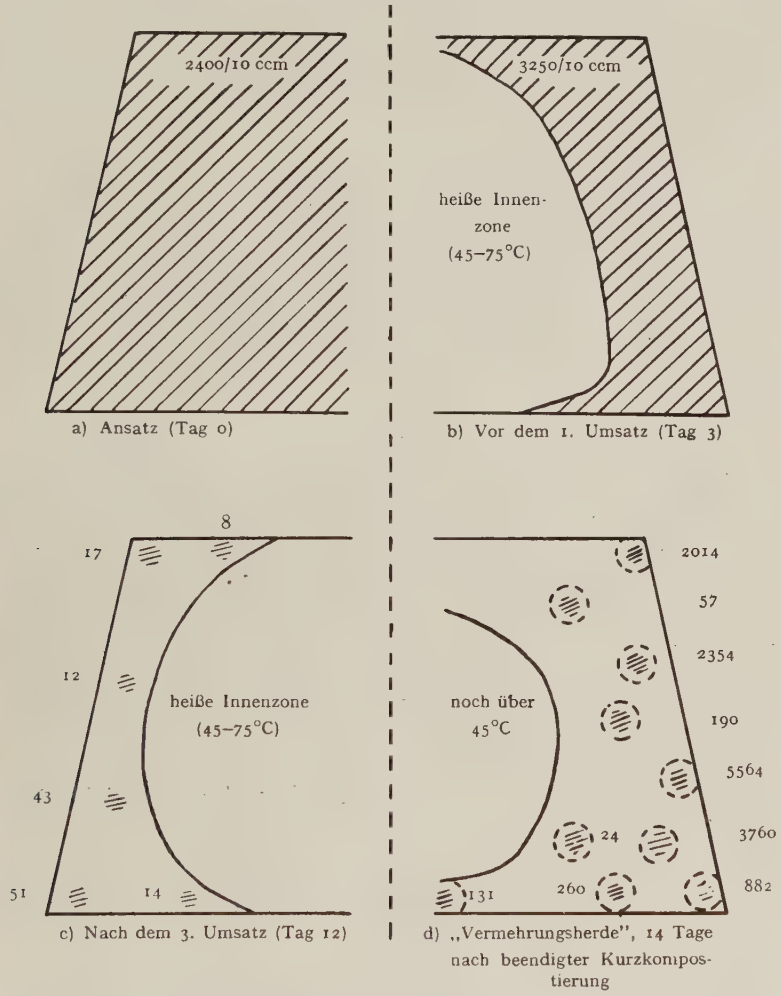


Abb. 2. Verhalten der Rhabditiden im Verlaufe der Kurzkompostierung.

weil zahlreiche Eier, möglicherweise auf Grund der grösseren Feuchtigkeit und der höheren Temperatur, ausgeschlüpft waren. Bei sorgfältigem Umsetzen des Düngers von Hand kann die Aussenzone fast vollständig in das Haufeninnere gebracht werden, so dass gegen Ende der Kompostierung nur noch relativ wenig Tiere fleckenweise über die Aussenschale verteilt sind (Abb. 2c). Lässt man den Kompost-

haufen aber noch einige Zeit nach beendigter Präparation stehen, so können sie sich z.B. in 14 Tagen um das 50- bis 100-fache vermehren. Dabei treten meist lokalisierte „Vermehrungsherde" auf (Abb. 2d), von denen die Neubesiedlung des Haufens ausgeht. — Synthetische Komposte erreichen oft weniger hohe Temperaturen und können daher am Ende der Präparation noch immer stark verseucht sein. Wenn man den Dünger mit Kompostmaschinen umsetzt werden ebenfalls weniger Nematoden abgetötet, weil die Aussenschale nicht vollständig nach innen kommt.

Eine endgültige Vernichtung der Nematoden ist nur durch eine Erhitzung des Kompostes auf 56-58° C im Anschluss an die Kompostierung möglich. Ohne eine derartige Behandlung ist die Champignon-Ernte von dem zufälligen Verseuchungsgrad des Düngers abhängig. Selbst stark mit *Rhabditiden* verseuchter Kompost, auf dem keine Champignons mehr gediehen, konnte durch Abtöten der Tiere regeneriert werden.

D. DER EINFLUSS DER RHABDITIDEN AUF DAS CHAMPIGNON-MYZEL

In den Beeten ist das Myzel an den fruchtkörperfreien Stellen grobsträngiger geworden oder fehlt ganz. Ein ähnliches Schadbild kann man in Flaschen-Kulturen beobachten (Tafel XV): Das Myzel wurde hier auf nematodenfreiem Kompost gezogen und später mit bestimmten aus Champignon-Beeten isolierten *Rhabditis*-Arten und Bakterien beimpft; die Kontrollflaschen nur mit Bakterien. Nach einer genügend starken Vermehrung der Nematoden verschwanden auch hier zunächst die feinen Hyphen und schliesslich auch die gröberen Hyphenstränge, während in den Kontrollen das Myzel erhalten blieb. Die Beobachtungen von Rempe & Kux, dass Myzel in Petrischalen (auf Malzextrakt-Agar) längere Zeit keine Schädigung zeigt, dann aber plötzlich innerhalb weniger Tage zusammenbrechen kann, treffen zu und deuten, wie die Autoren auch angeben, auf die Wirksamkeit von Stoffwechselprodukten hin.

Weitere Versuche in Petrischalen zeigten, dass eine besonders enge Assoziation zwischen *Rhabditiden* und Bakterien besteht. Die Nematoden verbreiten nicht nur, wie bekannt, die Bakterien über das Substrat, sondern scheiden vermutlich auch Substanzen aus, die die Entwicklung einer reichen Bakterienflora bedingen. Die Bakterien wiederum sind lebensnotwendig für die *Rhabditiden*. Bei unzureichender Ernährung mit Bakterien wird die Vermehrungsrate der Saprobionten stark herabgesetzt; ohne Bakterien werden die Larvenformen, wie auch Hirschmann (1952) feststellte, nicht geschlechtsreif und

schliesslich sämtliche Tiere inaktiv. Da die *Rhabditiden* auf Bakteriennahrung angewiesen sind, gelang es mir bisher nicht festzustellen, welchen Anteil sie an dem Myzelschwund und der Bildung von Schadstoffen haben. Grössere Versuche in Champignon-Beeten zeigten, dass die im Kompost vorhandenen Bakterien normalerweise nicht imstande sind, die Fruchtkörperbildung in einer derartigen Weise zu verhindern; erst bei künstlicher Infektion mit *Rhabditiden* kam es dazu.

E. ABHÄNGIGKEIT DER NEMATODENDICHTE VOM ALTER DER KOMPOSTE

Bei der Frage, ob Pflanzen durch Kompostdüngung von den Saprobionten geschädigt werden können, ist es notwendig zu wissen, zu welchen Zeiten die Tiere in grösseren Mengen verhanden sind.

In Pferdemist- oder Strohkomposten können die *Rhabditiden* nach etwa 6 Monaten ihr Maximum mit Durchschnittswerten von 6.000 bis 10.000 Individuen/10 cm^3 Kompost erreichen. Nach 15 Monaten sank die Populationsdichte im Mittel auf etwa 2260/10 cm^3 ab. Ältere Komposte dieser Art liegen z.Zt. noch nicht vor. Verschieden alte Komposterden aus Gärtnereibetrieben verhielten sich bezüglich der Nematodendichte ganz ähnlich (Abb. 3): In den 1-2 jährigen Komposterden waren noch 1830 Nematoden/10 cm^3 enthalten. Der letzte starke Abfall der Populationsdichte erfolgte in den 2-3 jährigen Komposterden. Hervorgerufen wird der starke Rückgang vor allem durch die Abnahme der *Rhabditiden*. Während nämlich in den 1-2 jährigen Komposterden noch 70-90 % der Nematodenfauna aus *Rhabditiden* bestehen kann, sind in den 2-3 jährigen Erden nur noch 40-50 % vorhanden, in den älteren nur noch 20 %, und schliesslich fehlen die *Rhabditiden* fast ganz. Sie werden von solchen Formen abgelöst, die allgemein in stärker abgebauten Substraten gefunden werden. Im allgemeinen ist das Artenspektrum variabel. Aber immer beobachtet man, dass mit zunehmendem Alter (Abbau) der Komposte die Nematodenfauna zahlenmässig rasch abnimmt und die anfangs sehr stark vertretenen *Rhabditiden* den stärksten Anteil am Rückgang der Populationsdichte haben; ihre Stelle wird von typischen „Erdformen" eingenommen. Die älteren Komposterden sind artenarm.

Die Aufeinanderfolge bestimmter Nematodengruppen dürfte auf ihre Nahrungsansprüche an bestimmte Abbaustufen des Kompostes zurückzuführen sein.

Die Untersuchung verschiedener alter Komposte zeigt also, dass grössere Mengen saprober Nematoden in 0-1 jährigen und in 1-2 jährigen Komposten zu erwarten sind. Kulturversuche, in denen

grüne Pflanzen mit derartigen Komposten gedüngt werden, sind gegenwärtig angelaufen. Eine Beeinflussung der Pflanzen wäre denkbar durch die Stoffwechselprodukte der saproben Nematoden und der von ihnen stark geförderten Bakterienflora. Ausserdem entziehen

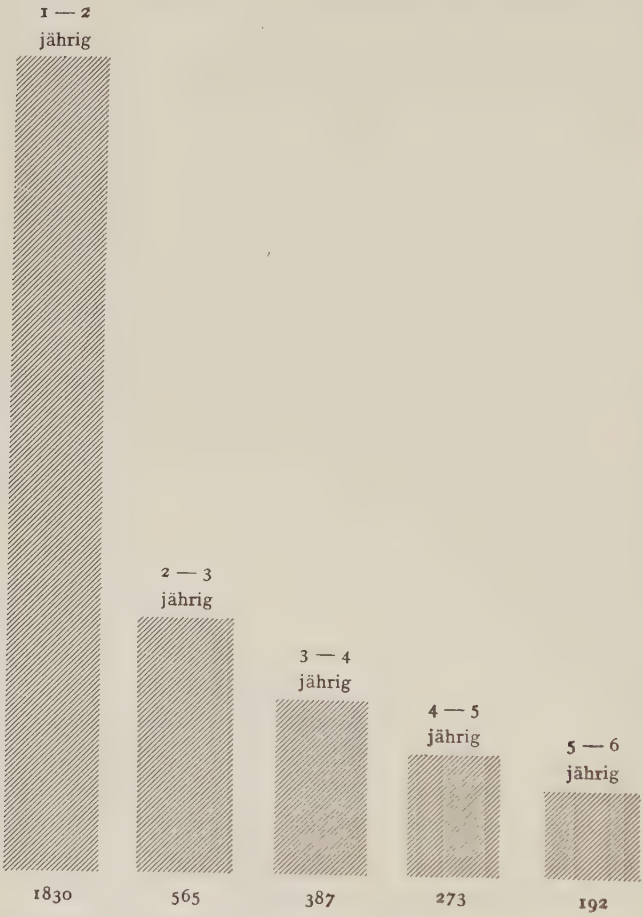


Abb. 3. Die Abnahme der Nematoden in verschieden alten Komposterden. (Gärtnerei-Komposten), Nematoden/10 ccm, Mittelwerte aus 50 Einzelbestimmungen je Komposterde.)

die Saprobionten über ihre Nahrung bestimmte Stoffe ihrer Umgebung. Da sie im wesentlichen Bakterienfresser sein dürften, würde ihre Nahrung zu etwa 50 % aus Eiweissen, zu 25 % aus Kohlehydraten und zu 25 % aus Fetten bestehen. Bei einem Massenauftreten der Tiere, z.B. in strohgedüngten Parzellen, würde also u.a. ein beträchtlicher Teil des Eiweisses in Nematodenkörpern festgelegt und für längere Zeit der Pflanze entzogen.

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DISKUSSION

J. H. Schuurmans Stekhoven: Ich würde bevorzugen von Saprozoiten zu sprechen, als von Saprophyten. Statt von Fermenten soll vielleicht eher von Exkretionsprodukten gesprochen werden.

Fr. Sprau: Wie Schuurmans Stekhoven halte auch ich es für richtiger nicht nur von Fermenten bei den Ausscheidungen der Nematoden zu sprechen, sondern ganz allgemein von Stoffausscheidungen, die die Entwicklung des Champignonmyzels hemmen oder es abtöten.

H. van Haut: Der Ausdruck „Saprophyten" ist in der Tat unrichtig. Im übrigen wurden aber die Bezeichnungen „saprobe Nematoden" bzw. „Saprobionten" gewählt. Ich halte den Ausdruck „saprobionte Nematoden" („Saprobionten") für zweckmässig.

Das Wort „Fermente" wurde irrtümlicherweise in der Ihnen vorliegenden Zusammenfassung benutzt. Im Vortrag selbst wurde von „Schadstoffen" gesprochen. Es ist aber noch ungeklärt, welchen Anteil die Saprobionten (neben den Bakterien) an der Bildung schädlicher Stoffe und damit am Myzelschwund haben; die saprobionten Nematoden fördern ja auch, wie erwähnt, das Bakterienwachstum.

M. Oostenbrink: In this country, the Netherlands, compost with which difficulties are met, normally contains a *Ditylenchus* species which is very close to *D. destructor,* together with other species. — The highest infestation rate we came across up till now was 1002 *Ditylenchus* and 839 saprosoitic eelworms per cm^3 of compost. The presence of *Ditylenchus* however not necessarily excludes the role of the saprozoitic eelworms in connection with the growth of mushrooms.

H. van Haut: Da *Ditylenchus* das Myzel direkt angreift, dürften bereits geringere Mengen dieser Art sehr schädlich sein und die anwesenden *saprobionten* Nematoden und die Bakterien den Zerfall des Myzels noch beschleunigen. — In den Ausgangsmaterialien der Komposte (Pferdemist oder Stroh) fand ich meist keine *Tylenchiden,* wohl aber in der sogen. Deckerde, mit der der Kompost in den Beeten überzogen wird. Ich möchte daher annehmen, dass *Ditylenchus* in den holländischen Kulturen mit der Deckerde eingeschleppt wurde, zumal öfters sandige Böden oder auch Gartenerden zum Abdecken der Beete benutzt werden.

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NOMENCLATURE

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Change of Address:

Bijloo, J. D. has left the Research Team for Control of the Potato Root Eelworm (T.N.O.), Wageningen, *Now*: Agro-Biologisch Laboratorium „Boekesteyn", N.V. Philips-Roxane, 's-Graveland (The Netherlands).

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THE MELOIDOGYNE PROBLEM IN SOUTH AFRICA

BY

W. J. VAN DER LINDE

At the last Nematological Conference there was mixed feeling as to the validity of CHITWOOD's splitting the old species, *Heterodera marioni,* into a number of different species. I think the nematological world owes Dr. CHITWOOD a vote of thanks for having taken the initiative. Those workers who today are studying root-knot nematodes realise the necessity for the creation of the different species of *Meloidogyne.*

In the past it was most difficult to prescribe any crop rotation for the control of nematodes, for none of the prescribed systems were ever really effective. Today the picture is somewhat different, for although not by any means an easy task, we at least understand the problem a good deal better. We can at any rate make a survey of any particular cultivated area and establish what species are present in that area; should crop rotation prove to be the only means of combating the nematode in such an area, an attempt can be made with some certainty to prescribe the crops that should be grown in such a rotation.

In Southern Africa or Africa as a whole these nematodes are indigenous. They can be found in certain localities living on plants in the veld, but never becoming a pest of these plants; they only show up when an economic crop is grown.

Some of the species described are very distinct and relatively easy to distinguish morphologically and by host reactions. We in South Africa however experience great difficulty with certain geographical forms of *Meloidogyne* which differ relatively little on morphology and host reactions.

In our preliminary survey of the distribution of the different species of *Meloidogyne,* a number of forms were found in different localities which morphologically resemble *M. incognita* and *M. incognita* var. *acrita.*

The nematodes collected at Vaalhartz and Roodeplaat experimental stations, appeared to be *M. incognita,* but on applying the host reaction

test (SASSER, 1954), these parasitized *Lycopersicon peruvianum*. Thus according to these tests they must be *M. incognita* var. *acrita*.

The nematodes collected at Elandskop, Oribi and Underberg in Natal and Ceres and Berlin in the Cape, Kitwe in N. Rhodesia and Odendaalsrust in the O.F.S., all somewhat resemble *M. incognita* var. *acrita* morphologically, but it is difficult to establish their identity definitely. They however all parasitize *L. peruvianum* and they are thus regarded to be closely related to, though not necessarily identical with, var. *acrita*. All these forms which morphologically resemble *M. incognita* var. *acrita* do not however show much penetration and very little, if any, reproduction when tested against *Gossypium hirsutum*. Their actual identity is therefore uncertain.

A number of host susceptibility tests were then carried out to study the reactions of some of these forms towards host plants and to compare them with other well identified species, viz. *M. hapla, M. arenaria* subsp. *thamesi* and *M. javanica*.

Unfortunately some of the forms were only discovered recently and it was not possible to include them in all of the tests. When these forms were originally found, a single egg-sac from each female was inoculated in sterilized soil on the roots of a bean plant; but many females from the original plant material were examined to establish whether only one species was present. From the single egg-sac a large population was built up to carry out these tests.

The tests were carried out in a greenhouse, there were five replicates for each nematode species or geographical form. Each replicate was inoculated with 25 egg-sacs and the experiment continued over ninety days, to insure development and reproduction.

All forms were tried against peanuts, to ascertain whether this crop was susceptible to all these races, but the only infestation was obtained from the nematode collected on strawberries and identified as *M. hapla*. This was verified by the susceptibility of *Zea mays* to the other forms, but not to *M. hapla*.

We believe that we will be able to differentiate between these apparent *acrita* populations by means of host susceptibility. For example, the population from Elandskop was tested together with the populations from Vaalhartz, Underberg and Ceres against the grass *Panicum maximum*, Olifantsriver strain (Northern Transvaal) and it was the only one which reproduced in its roots. Again the population from Elandskop was tested with those from Vaalhartz and Underberg against *Pennisetum glaucum* and only they reproduced in its roots.

TABLE I

Host Susceptibility Tests with Meloidogyne spp, with indication of locality

Scientific name	Common Name	Horticultural variety	*M. arenaria* subsp. *thamesi* Premier Mine	*M. hapla* Brits	*M. incognita* var. *acrita* Vaalhartz.	*M. incognita* var. *acrita* Elandskop	*M. incognita* var. *acrita* Underberg	*M. incognita* var. *acrita* Berlin	*M. incognita* var. *acrita* Oribi	*M. incognita* var. *acrita* Odendaalsrust	*M. incognita* var. *acrita* Roode plaat	*M. incognita* var. *acrita* Ceres	*M. incognita* var. *acrita* Kitwe	*M. javanica* Rietrivier
Lycopersicon peruvianum L.	Peruvian wild tomato		+	+	+	+	+	+	+	+	+	+	+	+
Phaseolis vulgaris L.	Common bean	many varieties	+	+	+	+	+	+	+	+	+	+	+	+
Fragaria vesca L.	Strawberry	many varieties	—	+	—	—	—	—						—
Linum usitatissimum L.	Linseed		+	+	+	+	+	+						+
Arachis hypogea L.	Groundnuts	Natal common Virginia bunch	—	+	—	—	—	—	—	—	—	—	—	—
Ricinus communis L.	Castor oil	Many dwarf varieties (Commercial)	±	+	a b + c	a b + c	+	+	a b + c	a b + c	a b + c	+	a ± *	+
Capsicum frutescens L.	Sweet pepper	California wonder	—	+	+	+	+	+	+	+	+	+	+	—
Capsicum annuum L.	Red pepper		—	+		+	c +		+	+	+			—
Gossypium hirsutum L.	Upland cotton	Many varieties	a b +	+	? b + c	c ±	c ±	—	a ± c	c ±	c ±	—	c ±	c ±
Crotalaria incana L. *nubica* Benth. *longirostrata* Hook a. Arn					±	±	±	±						±
Crotalaria spectabilis Roth.	Sunnhemp		±	±	±	±	±	±	±	±	±	±	±	±
Crotalaria juncea L.	Sunnhemp		+	+	+	±	+	+	±	±	±	+	+	+
Stizolobium deeringianum Bort.	Velvet bean	Velvet somerset	b +	—	b +	b c +	+		a c ±	a b + c	+	+	±	+
		Velvet golden	+	—	—	—	+		±	+	+	+	a +	±	+
		Velvet stingless	—	+	—	—	+		—	a b + c	b +	a +	b +	+
Lycopersicon esculentum Mill.	Tomato		+	+	+	+	+	+	+	+	+	+	+	+
Lycopersicon esculentum Mill.	Tomato	52029 × C tomato cross	—	+	—									—
		52152 × A tomato cross	—	+	+									--
		52065 × A tomato cross	—	+	—									—
Glycine javanica L.					+	+	+	+						+

\+ Penetration and reproduction
— No penetration
± Penetration no reproduction

a Excessive males
b Not more than 5 egg-sacs in all 5 replicates
c Immature forms.
* In one variety one egg-sac with 20 eggs was obtained.

TABLE II

Locality:	*M. javanica* Rietrivier.	*M. incognita* var. *acrita* Vaalhartz.	*M. incognita* var. *acrita* Elandskop	*M. incognita* var. *acrita* Underberg	*M. incognita* var. *acrita* Ceres	*M. incognita* var. *acrita* Berlin	*M. hapla* Brits.	*M. arenaria* subspecies *thamesi* Premier Mine
Chloris gayana (Kunth.)	+		+	+		+		
Eragrostis curvula (Schrad.) Nees. (Ermelo)	–	–	–	–	–	–	–	–
Eragrostis curvula (Schrad.) Nees. (Witbank)	+		+	+		+		
Eragrostis curvula (Schrad.) Nees. (Grootfontein)	–	–	±	–	±	±		
Eragrostis lehmanniana Nees.	–	–	±	–	±			
Eragrostis tef (Zucc.) Trotter.	+	+	+		+			
Panicum maximum Jacq. (Olifantsrivier)	–	±	+	–	–		–	–
Panicum maximum Jacq. (Rhodesian)	–	–	–	–	–	–		
Pennisetum glaucum (L.) babala	–	–	+	–			–	–
Sorghum almum L.	+	+	–	–	+	–		
Sorghum vulgare Pers. (various strains)	+	+	+	+	+	+		
Eleusine indica (L.) Gaertn.	+		+	+		+		
Digitaria smutsii Stent. (various strains)	+		+		+	+		
Triticum aestivum L. wheat	+	+	+	+	+	+		
Setaria sphacelata (Schumach.) Stapf.	+	+	+	+	+	+		
Zea mays L. (various strains)	+	+	+	+	+	+	–	+
Ehrharta panicea	+	+	+	+	+	+		
Bromus inermis Leyss.	+	+	+	+	+	+		
Phalaris tuberosa L. (various strains)	+	+	+	+	+	+		
Oryza sativa L. rice	+		+	+			–	+

\+ Penetration and reproduction ± Penetration; no reproduction – No penetration

Furthermore the populations from Vaalhartz and Ceres reproduced in the roots of the grass, *Sorghum almum,* whereas those from Elandskop, Underberg and Berlin do not.

According to Table 1 there are slight differences in the susceptibility of the three varieties of *Stizolobium* to the *M. incognita* var. *acrita* populations; but these reactions are not significant enough to be of importance for differentiation purposes.

Since the above mentioned plants are of considerable importance in the Agriculture in South Africa, we believe it is desirable to continue with these reaction studies, in order to obtain a more definite picture of how these forms differ in their host reactions.

In addition to these tests a search for resistant host plants was made for the purpose of finding plants suitable to grow in rotations.

These results are contained in Table I and II. From these tables, although far from complete, certain interesting facts emerged. On a commercial variety of castor oil for example it was noticed, that although reproduction takes place in most cases many more males and young forms than adult females were observed. In the case of *M. arenaria* subsp. *thamesi* (Premier Mine) no reproduction took place, whereas *M. javanica* and *M. hapla* reproduced more freely than did the *acrita* populations.

From the Tables it can be seen that there is no difference in host reaction in the studies so far carried out between *M. arenaria* subsp. *thamesi* and *M. javanica* except in the stingless variety of the velvet bean. This result has yet to be confirmed in a further experiment.

With regard to the perineal patterns, *M. arenaria* subsp. *thamesi* resembles *M. hapla* rather than *M. arenaria.* It was only after testing it against groundnuts and maize that we established it as *M. arenaria* subsp. *thamesi,* since unlike *M. hapla* it reproduces in maize but does not attack peanuts and strawberries. *M. arenaria* subsp. *thamesi* like *M. javanica* does not parasitize red pepper (*Capsicum annuum*).

From the above can be seen, that we in South Africa attach great importance to comparative host reaction studies, for we feel that it is more advisable to control these nematodes by cultural rather than chemical means. The obvious reason for this is that we wish to encourage crop rotations wherever these can be practised whether root-knot nematodes are present or not. We are especially interested in the introduction of grass lays into our rotation systems since they build up the soil. From Table II will be seen that many grasses have been tested and that very few are resistant to all the recognised species

of *Meloidogyne* with which we have so far been concerned. The main problem in this country as regards crop rotation systems, is that farmers are generally only willing to include cash crops in a rotation. For instance a tobacco farmer is willing to include, wheat, maize and ground nuts in his rotation and lately cotton and castor oil have also been favoured as well; in certain areas potatoes and other vegetables have also been included. In the South African tobacco areas *M. javanica*, *M. arenaria* subsp. *thamesi* and *M. hapla* and the *acrita* complex, all occur, in many cases two different species or more in the same field. Any of the above crops are therefore unsuitable in a rotation, and it is with great difficulty that farmers can be persuaded to include *Eragrostis curvula* (Ermelo strain) or *Crotalaria spectabilis* (sunnhemp), in their rotations, since neither the grass nor the sunnhemp has any cash value; this, in spite of the fact that neither of these two plants permit reproduction of any of the above species of *Meloidogyne*. Again in our major seed-potato producing areas where potatoes are grown under irrigation, the main alternative crops are groundnuts, lucern and cereals; in these areas *M. javanica, M. hapla* and the *acrita* complex are present, once more making the rotational crops mentioned unsuitable as far as nematode control is concerned. Here again farmers are unwilling, on their relatively small irrigable holdings, to include *E. curvula* or *C. spectabilis* in a rotation. However, it must be stated that farmers are slowly beginning to realise the importance of controlling nematodes, and of doing so by means of resistant plants which have the additional advantage of improving soils. They are realising that *E. curvula* (Ermelo strain) is a good grazing grass and also makes good hay; also that *C. spectabilis* although not as easy to cultivate, is probably as good a green manure as *C. juncea*; unfortunately *C. spectabilis* seems to be more susceptible to *Fusarium* wilt.

Of course, in any cultivated field where only one species of *Meloidogyne* occurs, the question of crop rotations is comparatively simple. There are, for example, many cases in the tobacco areas where only *M. javanica* occurs: here groundnuts and *Pennisetum glaucum* can be included safely in this rotation, but we still encourage the use of *E. curvula* and *C. spectabilis*, both for their soil-improving characteristics as well as for the fact that the introduction of another species can occur so easily under irrigation. It should also be mentioned here that *Meloidogyne* species occur throughout the country and that it is not always possible under colder climatic conditions to cultivate

C. spectabilis, and as *E. curvula* (Ermelo strain) is the only other plant resistant to most species, it alone can be recommended.

Nematodes of the genus *Meloïdogyne* play such an important role in agriculture, that many attempts have been made to control it. Seed potatoes play an important part as a cash crop, and it is required that these be grown nematode free.

None of the soil fumigants have given sufficient protection even at a dosage of 60 gallons to the acre. The best results so far obtained by fumigation gave 87% clean seed. This figure is not good enough, for the inspection services demand 100% clean tubers.

Attempts were therefore made to kill the nematodes in seed potatoes by refrigeration at different temperatures 31°, 34°, 37°, 40°, 45°, 50° F and air temperature. Seed potatoes kept at 31° F for 6 months must be planted immediately since they decompose rapidly at air temperature, the larvae and adult forms are killed after one month. Some eggs still hatch after six months at this temperature, but the vitality of the larvae which hatch from these eggs is very low, and the infestation which results from these larvae is much lower than in the case of potatoes kept at higher temperatures.

The hatching of larvae out of eggs from tubers kept at 34° F is low, but not as low as from potatoes kept at 31° F; adult forms also die within one month.

The hatching of larvae out of eggs from tubers kept at 37° F, 40° F, 45° F and 50° F is influenced very slightly, but many females are killed at these temperatures.

Although this work will perhaps not have a great deal of economic importance it demonstrates that a large proportion of the infestation can be killed by means of refrigeration; it also demonstrates that although the nematode is used to high temperatures in South Africa, yet a temperature of 31° F for six months cannot altogether destroy the eggs (See CUNNINGHAM, 1936).

Because of the natural occurrence of root-knot species in a large percentage of South African virgin land, it is most difficult to grow 100% nematode free seed potatoes. It is therefore necessary to divide the land into acre blocks for harvesting purposes. When any such area is found to be lightly infested its potatoes are discarded, the infestation sorted out and the clean tubers placed on the table market. This is the only way that we in South Africa have had any hope of growing seed potatoes up to now.

Rotation experiments are in progress in which the grass *Eragrostis*

curvula, Crotalaria spectabilis, groundnuts, *Pennisetum glaucum* are utilized, the results of these experiments are being awaited with anxiety. A combined rotational and fumigation experiment will be started during the next season.

For the nurseryman, who grows fruit trees especially peach, apricots, plums and figs and ornamentals, root-knot nematode is a serious problem, because the inspection service here again demands 100% clean roots. Soil fumigation does not protect the plant for a long enough time, therefore new methods of control must be found.

A number of nematode infested peach trees were fumigated with methyl bromide, at a dosage of 2½ lbs. per 1000 cubic feet at 65° F, as follows:

Treatment 1: 15 trees for 4 hours; treatment 2: 15 trees for 6 hours; treatment 3: 15 trees for 8 hours; treatment 4: 15 trees for 12 hours.

The roots of the trees were kept in moist straw during fumigation and sent from Cape Town to Pretoria by passenger train, after treatment. They were planted in drums in sterilized soil and examined 9 months later. The results are as follows:

Treatment 1: New roots were reinfested; treatment 2: New roots were reinfested; treatment 3: Three trees died, the new roots were clean; treatment 4: All the trees died, as a result of the Methyl bromide fumigation.

The above experiment was run during the winter of 1950 and the results obtained during May of 1951. Unfortunately the species of *Meloidogyne* were not determined.

A preliminary susceptibility experiment with a rose thornless rootstock proved that *M. hapla* infested the roots but not *M. arenaria thamesi* or *M. javanica.* The other populations were not tested.

A great number of fumigation experiments were carried out with D.D. and ethylene dibromide 40%; Ethylene dibromide proved to be the more effective soil fumigant on a price basis — i.e. half the quantity of ethylene dibromide proved as effective or more so, than D.D.

In conclusion it may be stated that up to 1950 a large amount of work had been done on the assumption of a single species of root-knot nematode. This work is to a large extent of little value, for susceptibility alone proves that we are dealing with a number of organisms of diverse habits and for each of which an economic solution has to be found.

In South Africa we have only started on the long journey, for nematode material was so far only collected from a few isolated spots. A small number of workers have to carry out the work over a tremendous area, with many different crops and climatic variations. A survey of the nematodes has to be carried out and control measures for each area have to be worked out.

SUMMARY

The need for creating the different species of *Meloidogyne* is briefly mentioned. Variations with regard to some host reactions within the *M. incognita* var. *acrita* group is discussed.

The similarity with regard to host reaction of *M. arenaria* subsp. *thamesi* and *M. javanica* is pointed out.

Crop rotational control measures are briefly discussed; results of a refrigeration experiment carried out with potato tubers are given.

Growing nematode free seed potatoes is a very difficult problem in South Africa and soil fumigation is too expensive and does not give adequate control.

A fumigation experiment with methyl bromide and peach trees demonstrates that this matter must be further investigated.

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INFLUENCE DE LA LUMIÈRE SUR LE DEVELOPPEMENT DU NEMATODE DES RACINES, MELOIDOGYNE SP.

(Résultats préliminaires)

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INTRODUCTION

L'emploi de nématodes des racines comme organisme de test dans l'étude des nématicides est une routine établie grâce à la simplicité de la technique de leur élevage. Afin d'obtenir des plantes plus fortement infectées par ces nématodes des racines, plusieurs auteurs (Tyler, 1933; Smith 1950) ont prolongé la photopériode des plantes-hôtes à l'aide de lumière artificielle. Tarjan & Hopper (1953) ont été les premiers à expliquer la signification de la photopériode pour l'activité reproductive de *Meloidogyne incognita* (Kofoid & White) Chitwood. Ces deux auteurs ont démontré expérimentalement que la longueur de la photopériode n'avait aucun effet significatif sur le nombre total de femelles ni sur celui des masses d'oeufs lorsque les plantes avaient été exposées à une température moyenne de 19° C. Cet effet devenait parcontre très manifeste lorsque les plantes avaient été exposées à une température moyenne de 26,5° C.

Ces auteurs ont réussi à augmenter de 57 % le nombre total des femelles et des masses d'oeufs en prolongeant la photopériode sans toutefois pouvoir réussir à influencer l'index de reproduction. Tarjan et Hopper conseillent pour obtenir une grande quantité de masses d'oeufs de *Meloidogyne* dans un laps de temps minimal d'élever les plantes inoculées à une température élevée et de prolonger la photopériode.

C'est dans ce même but que nous nous sommes proposés d'entamer l'étude sur l'influence de la lumière de différentes longueurs d'onde sur l'activité reproductive des nématodes des racines dans l'espoir de trouver la ou les fractions du spectre qui favorisent leur développement.

Les expériences préliminaires que nous avons entreprises n'ont pas la prétention d'avoir résolu ce problème de corrélation entre la

plante-hôte et ses parasites. Nous avons plutôt voulu poser le problème. Ce problème de corrélation nous paraît particulièrement intéressant puisque le facteur lumière, à l'encontre des facteurs température et humidité, n'influencent pas directement les nématodes vivant sous terre. Mieux nous connaîtront les relations de la plante-hôte et de ses parasites, mieux nous serons à même de trouver un moyen d'entraver le développement de ces nématodes.

La physiologie végétale nous apprend que la chlorophylle, élément photosensibilisateur responsable pour la photosynthèse, possède une absorption maximale dans la partie rouge (680 μ) et bleu-violette (460 μ) du spectre. Les rayons lumineux jouent non seulement un rôle important dans la photosynthèse mais également dans la mobilisation des auxines dans la plante (notamment dans les jeunes boutons, les jeunes feuilles et les bouts de racines). Les rayons rouges favorisent l'élongation des cellules; les rayons bleus nous procurent des plantes plus basses et plus trapues. Les rayons lumineux influencent la structure, le métabolisme et le chimisme des plantes. Dans quelle mesure une modification des facteurs précités sous l'action de rayons lumineux de différentes longueurs d'ondes pourrait-elle influencer éventuellement le développement des nématodes des racines? Voilà la question que nous nous sommes posée.

TECHNIQUE

Nous avons entrepris deux séries d'expériences préliminaires. Dans la première série d'expériences 30 plantes de tomates, âgées de 5 semaines, ont été inoculées avec 5 masses d'oeufs de *Meloidogyne arenaria*. Le jour même de leur inoculation (3 mars 1955) ces plantes furent mises dans des cages (Pl. XVI, fig. 1-2) éclairées par des tubes fluorescents (975 lumen) et installées dans une petite serre chauffée. La température, la même pour toutes les cages, oscillait entre 23° C et 28° C. Les quatre cages que nous avons employées étaient respectivement illuminées par des lampes rouges, vertes, bleues et blanches (A.C.E.C.).

Le graphique suivant (fig. 1) nous montre la répartition spectrale énergétique relative des tubes employés.

Les lampes LF vert-Emeraude et LF Rouge-Camélia sont les plus intéressantes pour des recherches pareilles vu l'implitude minimale de la courbe qui leur donne un spectre plus pur. La lampe bleue contient encore beaucoup de rayons verts et jaunes et est moins spécifique que les deux précédentes. La dénomination dans le texte de rayons

rouges, verts ou bleus, indique donc la lumière projetée par des tubes rouges, verts ou bleus. Il est plus que probable que des lampes ne donnant que des rayons rouges, verts ou bleus agiraient tout autrement sur les plantes et peut-être sur les parasites. Chaque cage contenait 6 plantes de tomates en pots. Trois d'entre elles recevaient la lumière

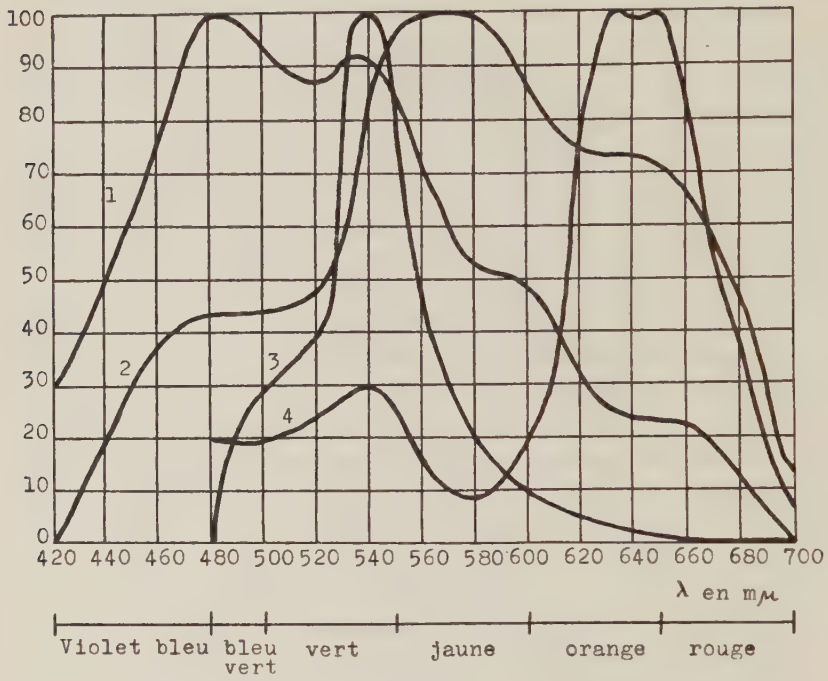

Fig. 1. Répartition spectrale énergétique relative des tubes employés.
1 = tube bleu 3 = tube vert
2 = tube blanc 4 = tube rouge

artificielle de la cage à partir de 3 h jusqu'à 23 h. Les trois autres furent soumises au même traitement avec cette différence toutefois qu'elles recevaient la lumière du jour de 9 h jusqu'à 17h 30. La distance entre les tubes et le sommet des plantes était de 32 cm. Un groupe de 6 plantes ne recevait que la lumière du jour (9 h-17h 30). Après un séjour de 6 semaines dans ces cages, les plantes furent levées, les racines coupées, débarassées des débris de terre et mises dans du formol de 5 %. Les racines de chaque plante furent complètement analysées quant au nombre de femelles, de grandes galles et de petites galles. Par le terme „grande galle" il faut comprendre une

Fig. 1. Vue extérieure des cages.

Fig. 2. l'Intérieur d'une cage.

galle formée par une femelle adulte avec ou sans sac d'oeufs, par „petite galle" celle causée par les divers stades de larvae.

Dans la deuxième série d'expériences 36 plantes de tomates âgées de 2 mois, furent inoculées au moyen d'environ 115 oeufs de *Meloidogyne arenaria* par plante. Ce n'est que 4 jours après l'inoculation (notamment le 9 mai 1955) que les plantes furent mises dans les cages et soumises au même traitement que celles de la première expérience. Une grande partie des larves écloses devait donc déjà se trouver à l'intérieur de la plante. Après 38 jours dans ces cages, les plantes furent levées et le nombre de petites et grandes galles, de femelles avec et sans sac d'oeufs fut déterminé.

RÉSULTATS

Les résultats de ces recherches préliminaires ne peuvent être considérés que comme provisoires vu le nombre limité de plantes examinées. Ils n'ont de valeur qu'à titre indicatif.

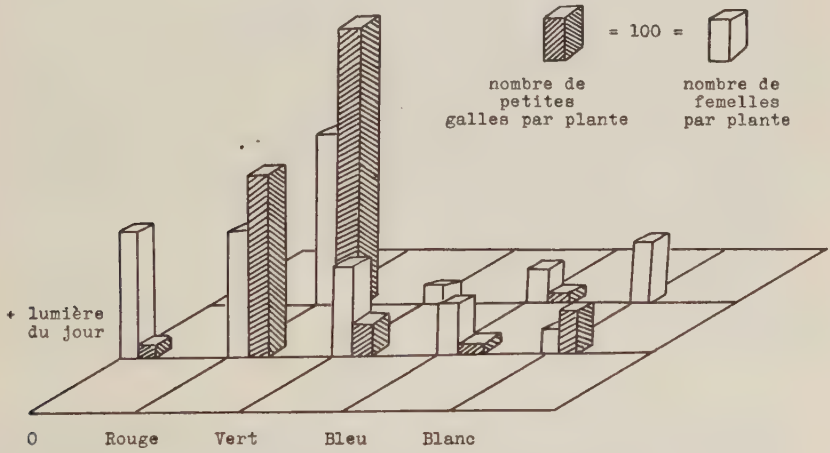

Fig. 2. Influence de la lumière de différentes longueurs d'onde sur le nombre de petites galles et de femelles par plante (explication voir texte).

Le graphique (fig. 2) nous donne un aperçu schématique des résultats de notre première série de recherches. Chaque colonne striée représente le nombre moyen de petites galles par plante. Les autres colonnes représentent le nombre moyen de femelles par plante. Toutes les colonnes de la première rangée ont reçu la lumière du jour. La première n'a reçu que celle-ci, les suivantes ont reçu pendant la nuit respectivement de la lumière rouge, verte, bleue et blanche. La

seconde rangé a reçu uniquement les lumières précitées. Le nombre de petites galles, contenant donc les larves de divers stades, est toujours moins élevé que celui des femelles avec ou sans sac d'oeufs, les groupes rouge et blanc exceptés où les proportions sont inverses. Chez les plantes „rouges" le nombre de petites galles dépasse largement celui de tous les autres groupes. Il est plutôt difficile d'expliquer cette constation. Faut il attribuer ce grand nombre de petites galles aux larves d'une deuxième génération? Dans ce cas il faudrait admettre que les rayons rouges aient stimulé et hâté le développement des nématodes. Ou fait-il imputer ce grand nombre de petites galles ou fait que les plantes „rouges" auraient attiré les larves plus fortement que les autres plantes?Dans ce cas il faut admettre que la lumière rouge ait influencé la quantité ou la nature des sécrétions radiculaires attirant les larves.

Les résultats de notre deuxième série d'expériences ne nous permettent pas de conclure à une nette influence de nos tubes rouges, verts, bleus et blancs sur l'index de reproduction.

Dans cette deuxième expérience la plus grande partie des larves se trouvait déjà à l'intérieur des racines lorsque les plantes furent mises dans les cages éclairées. Il se peut que la longueur d'onde n'influence plus les nématodes une fois que celles-ci sont entrées dans les racines. L'hypothèse que les rayons rouges favoriseraient l'attraction des nématodes vers les racines nous semble donner une explication provisoirement satisfaisante.

Des recherches ultérieures et plus approfondies tacheront d'élucider ce problème de corrélation entre la plante-hôte et les nématodes des racines.

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BEET STEM EELWORM

BY

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Stem eelworm, *Ditylenchus dipsaci* (Kühn) Filipjev, has been known for some time to attack beet and mangolds, producing a crown canker in the autumn (GOODEY, 1929; ROEBUCK & HULL, 1937; RIETBERG, 1953). The first cases of it causing damage to seedling beet and mangolds in the field were noted in England in the spring of 1953 (DUNNING, 1954 a, b.).

Infestation of the seedlings causes bloating and galling of the leaf midribs and petioles, with very variable thickening and distortion of the leaves themselves. Should invasion of the growing point occur, which can happen at any time between the cotyledon stage and the 4-6 rough leaf stage, the growing point becomes stunted and malformed, whilst the outer leaves become thickened and contorted. Subsequently, fresh growing points develop from the axillary buds, leading to a multiple crown condition with elongated leaves and slight distortion. During the summer, healthy growth is produced on both single and multiple crown beet, and evidence of infestation is no longer apparent.

The race concerned in both spring and autumn infestation is the general field race, which attacks oats, rye, onions, etc. In addition, however, beet stem eelworm (crown canker material) infested and reproduced in sainfoin (*Onobrychis sativa*), alsike (*Trifolium hybridum*), and wild white clover (*Trifolium repens*), producing typical symptoms. The seedlings were severely stunted and some died, but the remainder eventually 'grew away' from the attack. Eelworms of the red clover race invaded beet seedlings and produced malformation, but did not breed, confirming previous work done in Holland (in litt.).

In the field, attacks have occurred on beet grown in onion stem eelworm infested soil, and on mangolds grown after frequent cropping

of beet and oats, but the source of infestation of some of the worst cases seen on beet in 1953 was not clear. In infested fields, little evidence can be seen of the patchiness so characteristic of stem eelworm attack, and infested plants are to be found among apparently healthy plants. It appears that beet is not very susceptible to stem eelworm attack; beet grown on a patch of fen soil where onions were devastated the previous year showed less than 20% infestation of the seedlings, infested seedlings being scattered among healthy ones. On the other hand, in 1953, some 30-40% of beet were infested in a field with no previously known stem eelworm infestation. Although attacks on beet are rarely severe, they are widespread. In a survey of beet fields in Nov.-Dec. 1954, near Cambridge, England, 22% were found to be infested, mainly very slightly. Field trials in progress at present indicate that mangolds are more susceptible than beet to stem eelworm infestation.

Eelworms can be carried on the seed, but this is of little or no practical importance — few samples of seed contain any eelworms, and then only in very small numbers. When seed infested with eelworms at the rate of 100/cluster was sown, the percentage of infested seedlings resulting was almost nil.

Autumn crown canker was previously thought to arise by invasion of the 'root' at soil level in summer and early autumn, but the present work has shown that spring infestation of the petioles, leading to infestation of the leaf scar region, is an additional, and probably the more usual, means of invasion. The canker occurs mainly above soil level, especially in mangolds, and multiple crowned beet can invariably be found in fields where stem eelworm canker occurs, both points indicating spring invasion. However, the exact relationship between spring and autumn infestation is not yet clear.

The progress of the eelworms and the canker in the root is very variable. In some the whole crown is cankered through in November, whilst in others it develops very slowly indeed, even if the roots are kept until the following spring. Usually the canker starts at the lowest leaf scars as a slight lesion, which later becomes raised. If the canker progresses, the area becomes sunken and cracked.

In England, beet stem eelworm has so far rarely caused economic loss, but it is reported as a serious pest in Switzerland (Savary, 1953). The importance of beet and mangolds as hosts of stem eelworm should not be underestimated.

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ROOTROT OF BULBS CAUSED BY PRATYLENCHUS AND HOPLOLAIMUS SPP.

BY

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In Holland, the bulb-growing area is extensive and embraces a variety of soils, viz: — sand, sand mixed with clay or peat, clay, and peat. Rootrot of bulbs and corms caused by nematodes may be found on all the above soils, except on clay. Rootrot is most feared in nurseries and those parts of the bulb-growing area where bulbs, susceptible to nematodes, are cultivated continuously. Here Pratylenchidae are most prevalent. Double digging to a depth of 24 in. (0.6 m.), a normal practice for tulips, is useless for the control of eelworms; digging to 36 in. (0.9 m.) or more is more satisfactory. In some localities, besides bulbs, other crops are grown in the nurseries, such as vegetables, flowers, strawberries and cereals, oats and maize being excellent hosts for Pratylenchidae. *Pratylenchus* or *Hoplolaimus* spp. do not enter bulbs and so it is impossible to transmit the disease by bulbs with well-dried roots (e.g. narcissi). Rootrot is also difficult to transmit by movement of soil.

The incidence of rootrot varies from year to year. Field and laboratory experiments have shown that heavy rainfall when there is no host plant growing (e.g. between the lifting of bulbs and root formation of the following crop) reduces the nematode population. Small populations of the order of ten Pratylenchidae per kilogram of soil can cause serious rootrot in narcissi. This appears to be related to the small number of roots formed by bulbs, which often are not replaced during the growing season, and also to the fleshy nature of the roots and their high susceptibility to injury by secondary fungi. Susceptibility varies from species to species, gladioli and montbretias are non-susceptible while narcissi and lilies are susceptible.

The best known rootrot disease in the bulb-growing area is that of narcissi which was first reported 40 years ago. Where the soil is unsuitable for growing hyacinths, narcissi are frequently planted and

formerly it was not uncommon for them to be grown in the same fields for several years in succession. Where crops were rotated, narcissi were grown once every two or three years. Formerly, two crops of narcissi were often taken in succession, especially certain varieties such as *Narcissus exertus* (Haw.) Pugsley var. *ornatus* Pugsley. In the second year severe rootrot was liable to appear. Rootrot of narcissi seldom occurs in nurseries where hyacinths are grown regularly. This is not because narcissi are not grown, but because the soil is regularly disinfected with formaldehyde to destroy *Fusarium culmorum* (Sm.) Sacc. and this treatment also reduces the population of *Pratylenchus*.

In the autumn, the nematodes begin to attack the newly formed narcissus roots. Typical small, longitudinal, striped lesions are formed which enlarge until considerable areas of root surface are rotten. Finally, the whole root system decays. In the spring, the above-ground symptoms of rootrot are distinctive and appear as patches of stunted plants where the soil population of *Pratylenchus* is greatest. The plants wilt and die in warm sunny weather long before lifting time. In the patches, the bulbs have made little or no growth but, if planted in nematode free soil the next year, they give a normal crop.

Hastings, Newton and Steiner (1932) supposed that *Tylenchus pratensis* De Man was the primary cause of this root decline. Hodson (1939) presumed that "root eelworms" were the cause of rootrot of narcissi in England. Jensen (1953) named *Pratylenchus* spp. as the cause of rootrot of narcissi in Oregon (U.S.A.) and afterwards identified the causal agent as *Pratylenchus penetrans* (Cobb) Sher & Allen. Meanwhile in Holland several investigators put forward various agents as the cause of root rot in narcissi. Feekes (1931) stated that the fungus *Cylindrocarpon radicicola* Wr. was responsible, while v. d. Meer (1952) supposed that unfavourable soil aeration (zones of reduction of organic matter in which H_2S is produced) was responsible.

EXPERIMENTS DEMONSTRATING THE RÔLE OF P. PENETRANS IN NARCISSUS ROOTROT

When investigation of rootrot was started at Lisse, the rôle of *Pratylenchus penetrans* (Cobb) Sher & Allen in the production of rootrot was questionable. It was necessary to determine whether this nematode was the cause or merely a secondary invader. As it was not possible to culture large numbers of Pratylenchidae *in vitro* without

contamination by soil fungi, alternative procedures were necessary. Hastings and Bosher (1939) mixed root inoculum with peaty soil soaked in a solution of Malachite Green. Healthy seedlings were sown in the mixture and, when moving from the inoculum to the seedling roots, the nematodes were disinfected by the chemical and the secondary fungi suppressed. In our experiments Malachite Green did not give satisfactory results and attempts were made to find other chemicals with analogous effects without injuring the nematodes. Tetramethylthiuramdisulphide (TMTD) at 20 g./kg. soil proved to

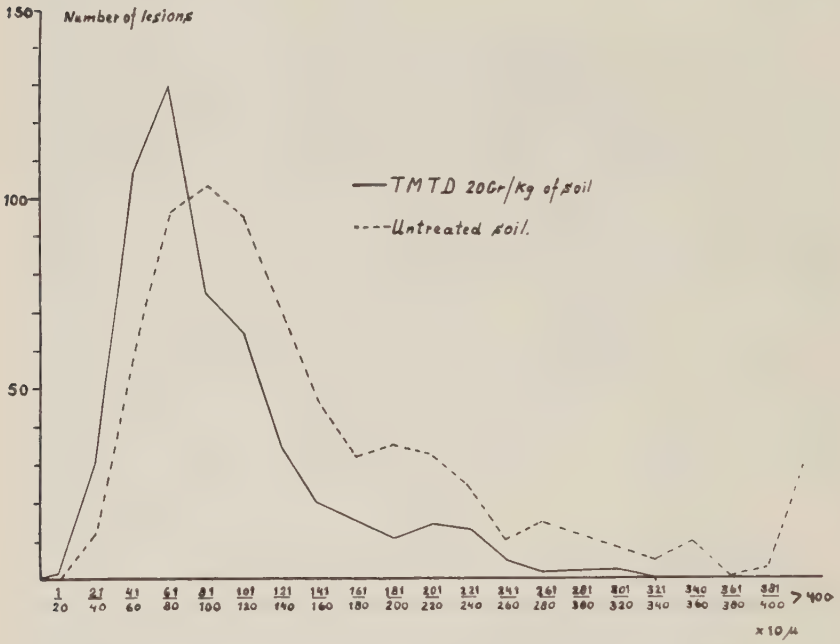

Fig. 1. Size of the lesions two months after planting the bulbs.

be the best inhibitor of fungi and did not injure the sensitive Pratylenchidae. In this soil the number of lesions upon narcissus roots was scarcely affected compared with controls, but the size of the lesions was reduced (Fig. 1). Microscopical examination showed that the lesions on roots grown in untreated soil contained much fungal mycelium in contrast with lesions on roots grown in TMTD treated soil which were relatively free. *Cylindrocarpon radicicola,* which is common in untreated soil, did not develop in cherry agar isolations from treated soil. Ferridimethyldithiocarbamate (Ferbam) gave the

same results but was rather toxic to nematodes. Comparable results were obtained with mercurial compounds. Soaking the soil with dilute Aretan (0.05%) or disinfection of the inoculum (0.5% for 20 min.) were effective but caused heavy losses of nematodes.

Further experiments were made on the rôle of nematodes in the *Pratylenchus penetrans — Cylindrocarpon radicicola* complex. To investigate whether *P. penetrans* is truly parasitic on narcissus roots or enters only through root injuries, bulbs were planted in boxes infected with a mixture of parasitic and saprozoic nematodes. After root formation, the hinged sides of the boxes were opened and portions of root from 5-8 cm. long were marked with Congo Red. Between the marks the roots were injured with a needle at several points and the boxes closed. The boxes were re-opened after two months with the following results (Table I).

TABLE I

Effect of artificial injury of narcissus roots upon invasion by P. penetrans

Number of artificially injured roots		50
Number of individual injuries (punctures)		283
Lesions confluent with injuries:		
containing *P. penetrans*	20	
without *P. penetrans*	9	
		29
Lesions isolated from injuries:		
without *P. penetrans*	237	
containing *P. penetrans*	8	
		245
Lesions adjacent to injuries:		
containing *P. penetrans*	64	
without *P. penetrans*	53	
		117

Because of the migratory habits of *P. penetrans,* it is normal to find typical lesions without nematodes. Table I shows that lesions are formed freely in uninjured roots and that the nematodes appear to prefer undamaged tissue.

In a second experiment, narcissi were planted in sterilised soil. Roots injured and infected with a pure culture of *C. radicicola* produced lesions similar to natural ones. In the absence of the fungus,

no lesions developed and in the absence of injury the fungus failed to penetrate into healthy roots.

In a third experiment, root inoculum was ground in a colloid mill for 1 hr. at 800 r.p.m. Microscopical examination showed that the spores of the fungus were undamaged. They were still capable of developing when poured on to cherry agar. In contrast, all nematode eggs were destroyed. Infections attempted with ground inoculum did not cause rootrot whereas the original inoculum did.

The three experiments outlined above justify the conclusion that *P. penetrans* is parasitic upon the roots of narcissi and provides the fungus *C. radicicola* with a means of entry into the roots.

ROOTROT CAUSED BY NEMATODES IN OTHER BULBS

On patches where narcissi exhibit symptoms of rootrot, succeeding crops also show symptoms of attack by *P. penetrans* but only if the soil populations are great. Damage to hyacinths appears if the land is not disinfected with formaldehyde, the plants being dwarfed and dying prematurely. The roots bear small lesions which do not develop until the roots become rotten as in narcissi. Crocusses may be attacked without showing foliage symptoms. If the eelworm population is very great, damage to the roots may cause wilting and the leaves become straw-coloured. This symptom is distinct from the brownish colouration of leaves in plants attacked by *Botrytis* spp. Tulips attacked by *P. penetrans* are retarded in growth and develop a ruddy colour at the end of the growing period. Numerous small lesions are often visible on the roots. The growth of gladioli is retarded and small, red-brown lesions are found on the roots. The roots of onions and shallots are not susceptible to attack by secondary fungi. The lesions caused by *P. penetrans* remain minute and are whitish or pale yellow in colour. Injured plants wilt long before lifting time. In some nurseries around Wassenaar and Voorschoten, where lilies of the valley (*Convallaria majalis* L.) are grown, the crop is very susceptible to rootrot caused by *P. pratensis* De Man. Narcissi are not a host of this nematode. Occasionally both species occur in *Convallaria* roots. Similarly, crocusses on Texel suffer from *P. pratensis* whilst on the same patches narcissi remain healthy. These field observations show that narcissi are not susceptible to *P. pratensis* which, up to the present, has not been found in their roots.

In studying nematodes from infected roots, great size variation is

often found within a population. Many nematodes do not fit the descriptions of species given by SHER & ALLEN (1953).

Rootrot of lilies (*Lilium speciosum* Thbg. var. *rubrum* Mast.) in Kennemerland causes growers much difficulty. For several years bulbs were grown on the same land. Unlike other crops, the disease becomes apparent only at the end of the growing season. Dwarfing does not occur but the crop yellows long before the normal time. The root system is severely attacked and old roots decay, while young roots bear numerous lesions which coalesce to form necrotic areas. Attacked plants often produce many adventitious roots from the base of the stem.

As with rootrot in narcissi, the disease was first ascribed to *Cylindrocarpon radicicola* (v. HELL, 1931). MCWORTHER & ANDERSON (1951) have reported rootrot in Croft lilies in Oregon caused by *Pratylenchus* spp. In many of the attacks of rootrot in Kennemerland the disease was caused by *Pratylenchus* spp., probably *P. penetrans*. This nematode also attacks strawberries in this area causing black rootrot (KLINKENBERG 1953) but whether in fact the same species attack lilies and strawberries, is unknown. In lilies the disease is confined to the roots; nematodes are not found in the bulbs.

It is difficult to explain why narcissi are not attacked by the nematode attacking lilies. There may be different races or even species at present indistinguishable morphologically from *P. penetrans*. The 'lily race' also attacks snowdrops (*Galanthus nivalis* L.) causing severe damage.

Rootrot in lilies is not always due to *Pratylenchus* spp. It may also be caused by the ectoparasitic nematode *Hoplolaimus uniformis* Thorne which causes heavy losses when present in populations of 500 per kilo of soil. Rootrot in other bulbous or cormous crops due to *Hoplolaimus* has not yet been recorded although injury to gladioli has been noted on a few occasions. Generally both nematodes occur together.

CONTROL MEASURES

Formerly, rootrot was difficult to control. Steam sterilisation was highly effective but too expensive, while formaldehyde was not satisfactory when the soil was heavily infected. Some growers plant Montbretias (*Crocosmia crocosmiflora* N. E. Br. var. *meteor* Hort. or *C. crocosmiflora* var. *fantasia* Hort.) on heavily infected patches and, immediately after this, narcissi showed better growth. In field experiments the following results were obtained (Table II).

TABLE II

The effect of growing montbretias and vegetables in infected land.

Previous crop	Increase or decrease in yield narcissus bulbs per cent of weight planted							
Sites:	(1)	(2)	(3)	(4)	(5)	(6)	(7)	Mean %
Vegetables	—26	— 9	—18	+27	—17	—32	—19	—13
Montbretias	+63	+47	+60	+64	+55	+32	+41	+52

The difference between results at different sites is probably due to variation in the level of infection. Very heavy infection reduces the yield to less than the planting weight. In comparison with good nematicides, the improvement in yield brought about by montbretias is insufficient and their cultivation is not profitable. Furthermore, they also are attacked by root-lesion nematodes and the success of the method depends on trapping the nematodes by lifting the crop.

Several nematicides have been tested against rootrot diseases e.g. Chloropicrin, Ethylene dibromide (EDB) and Dichloropropane-Dichloropropylene (Shell DD), all at 42 ml/sq. metre.

The best results were obtained with Chloropicrin but this soil fumigant is too expensive and too difficult to handle for outdoor use on a

TABLE III

Influence of soil fumigation on nematode population and yields of Lilium speciosum var. *rubrum.*

Experiment No.	254	256	264	266
Treatment (4-9-1952)	DD	no	DD	no
Soil samples (before planting) taken on	6-3-53	6-3-53	2-3-53	2-3-53
Pratylenchus spp. in 1500 gr./soil	0	149	0	31
Hoplolaimus uniformis in 1500 gr./soil	0	726	0	1255
Soil samples taken on	29-7-53	29-7-53	14-8-53	14-8-53
Pratylenchus spp. in 1500 gr./soil	1	160	1	66
Hoplolaimus uniformis in 1500 gr./soil	2	193	17	9002
In roots from 2 bulbs belonging to the sample				
Pratylenchus spp.	33	10230	19	60
Hoplolaimus uniformis	0	3	6	270
Total *Pratylenchus* spp.	34	10390	20	126
Total *Hoplolaimus uniformis*	2	196	17	9272
Increase in weight of bulbs at harvest time (in % of the planting weight)	+52 %	+12 %	+77 %	—11 %

large scale. Ethylene dibromide proved unreliable, although sometimes giving good results. Shell DD proved most satisfactory under a variety of conditions and is now used on a large scale in the bulb-growing area for control of rootrot in narcissi and lilies. Table III gives the

Fig. 1. Root rôt of narcissi, early stage (Laurens Koster).

Fig. 2. Lesions of *Hoplolaimus uniformis* on *Lilium speciosum* var. *rubrum*.

Fig. 1. Control of rootrot of narcissi (Laurens Koster).
Above: Soil treated with Chloropicrin.
Below: Untreated soil.

Fig. 2. Control of rootrot of narcissi (Helios).
Left: Untreated soil.
Right: Soil treated with Shell DD.

Fig. 1. Control of rootrot of *Lilium speciosum* var. *rubrum*.
Above: Bulbs from soil treated with Shell DD.
Below: Bulbs from untreated soil.

Fig. 2. Control of rootrot of narcissi (Laurens Koster).
Left: Narcissi after a preceding crop of African marigolds.
Right: Control, uncropped.

results of experiments with *Lilium speciosum* Thbg. var. *rubrum* Mast.

Apart from its lethal action against Pratylenchidae, DD favoured the growth of narcissi giving increase of 10% or more as a normal occurrence. Experiments showed that 'stimulation' of growth in narcissi is not due to the destruction of other nematodes. 'Stimulation' has not been observed with tulips. Although the use of Shell DD is increasing, its cost is a disadvantage.

It was discovered by accident that rootrot in narcissi was greatly decreased by the cultivation of African marigolds (*Tagetes erecta* L.). In a confirmatory experiment, marigolds sown in July were worked into the soil with a rotory hac at the beginning of October. The next year narcissi sown in beds so treated gave much better growth than in untreated beds, the yield of bulbs being doubled (Table IV). This improvement, which was observed equally when whole plants or plants without leaves were hoed in, or when plants were lifted, cannot be

TABLE IV

The effect of African marigolds on the nematode population and the yield of narcissi

	Control	Marigolds worked into soil	Marigolds lifted	Marigolds, roots only worked into soil	Soil fumigated with Shell DD
No. of *P. penetrans* per 1500 g. soil	25	2	—	—	0
In roots of sample of narcissi	632	213	—	—	39
Total	657	215	—	—	39
Increase in yield of bulbs	79 %	141 %	148 %	190 %	202 %

explained, since roots of marigolds are parasitized by *P. penetrans*. The improvement obtained was less than from soil fumigation with DD.

Oostenbrink & Slootweg (not yet published) showed that *Tagetes* reduces *Pratylenchus* populations to about one tenth of the level after other crops. It would be interesting to investigate further the possibilities of control by enemy crops such as *Tagetes* under conditions where DD fumigation is too expensive or impracticable because of adverse soil conditions.

SUMMARY

Rootrot caused by nematodes is widespread in Holland on bulbs and corms. It occurs most commonly in narcissi and affects sandy or peaty soils in the bulb-growing area. The primary cause of rot is *Pratylenchus penetrans* (Cobb) Sher & Allen but *Cylindrocarpon radicicola* Wr. plays a secondary rôle. Tulips, hyacinths, gladioli, crocusses and scillas are also affected. Bulbs and corms suffer more than other crops because of their restricted root production which enables relatively small nematode populations to cause severe rootrot. In the narcissus-growing district, lilies of the valley are attacked by *Pratylenchus pratensis* de Man. This nematode does not harm narcissus roots. Lilies of the valley are occasionally attacked by *P. penetrans*.

In Kennemerland, lilies of the valley suffer severely from rootrot with heavy losses. As with narcissi, the primary parasite is a nematode, *Pratylenchus* sp. or *Hoplolaimus uniformis* Thorne with *Cylindrocarpon radicicola* Wr. as a secondary agent. Many fields are heavily infected with both nematodes. Experiments suggest that *P. penetrans* consists of a series of subspecies or races.

Rootrot in bulbs is limited to the roots. The nematodes do not invade the bulbs and, therefore, it is impossible to transmit the disease with bulbs with well dried roots.

Infested soils are fumigated on a large scale with Shell DD which, besides controlling the nematodes, appears to 'stimulate' the growth of narcissi. African marigolds grown on infected soil reduce the *Pratylenchus* population considerably.

The author wishes to thank Mr. F. G. W. Jones for assistance with English.

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DISCUSSION

J. H. Schuurmans Stekhoven: asked for information about Urea.

A. F. G. Slootweg: Urea is a good nematicide, if used in great quantities but causes severe injury to roots if it is not washed out of the soil by sufficient rainfall.

J. B. C. Vekemans: M. Slootweg peut il donner une explication à l'influence du DD sur le rendement? Le même phénomène a été observé en Congo Belge sur *Tabac* and *Coton* sans qu'une explication puisse être donnée.

A. F. G. Slootweg: Besides nematodes, other microorganisms are killed, probably in fumigated soils there is more nitrogen available for the plants. It is not a question of control of other nematodes.

R. S. Pitcher: I should like to congratulate the speaker on his pioneering work in this difficult and important field of associations between nematodes and micro-organisms and to ask him to say something of the methods he has used to distinguish between the activity of the fungi and the nematodes.

A. F. G. Slootweg: 1) Fungi + Pratylenchus → colloïd mill → no symptoms (nematodes destroyed) 2) Fungi + Pratylenchus + TMTD → small lesions (limited fungal development) 3) In sterilized soil, roots wounded by glass needle:

a) + fungi → lesions.

b) no fungi → no lesions.

c) roots not wounded + fungi → no lesions.

The fungi are mainly *Cylindrocarpon radicicola.*

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AN INTERPRETATION OF SOME CROP ROTATION EXPERIENCES BASED ON NEMATODE SURVEYS AND POPULATION STUDIES

BY

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INTRODUCTION

This paper gives additional information about the occurrence of a parasitic nematode complex in cultivated land and its possible significance as a growth-inhibiting factor in crop rotation. The senior author has recorded earlier work on the role of root attacking nematodes in crop rotation effects (1954, 1956 a).

Soil which has been under cultivation for several years harbours plant-parasitic nematodes in addition to the saprozoic forms. This has been noted in other countries and is normal (see e.g. JENKINS, TAYLOR & ROHDE 1956). Under Dutch conditions root attacking nematodes of the genera *Heterodera, Hoplolaimus, Meloidogyne, Paratylenchus, Pratylenchus, Rotylenchus* and *Tylenchorhynchus* were most frequent. In a preliminary survey more than 70 different taxons of these genera were recorded, many of them undescribed. For at least 30 taxons, distributed throughout all the above genera, evidence is available that they act as true parasites in agriculture, and the number is expected to increase when the four last-mentioned genera are studied in detail. Cultivated soil commonly harbours a mixed population of several of these genera, with two or more species in each. The taxonomical part of this study was facilitated by the presence of a collection of permanent eelworm slides.

Heterodera cysts were washed from dried soil samples. Active nematodes were collected from moist soil. The extraction rate for different species is estimated to be between 50 and 90 %. Root samples were examined by a standardized funnel-spray-method, which furnishes representative data about infestation by *Pratylenchus* and other endoparasitic migratory species. A full survey of the techniques employed will be given elsewhere.

EFFECTS OF CROP ROTATION ON SANDY SOIL

Cultivated land in sandy areas normally harbours a complex of several species of root infesting nematodes, the density and composition of which fluctuates markedly with the crops.

TABLE I

Nematode populations on a farm on diluvial sandy soil.

Number of nematodes per 200 cc of soil, 1955/'56.

P. = *Pratylenchus*; Pa. = *Paratylenchus*; M. = *Meloidogyne* larvae; R. = *Rotylenchus*; H. = *Hoplolaimus*; T. = *Tylenchorhynchus*; O. = other *Tylenchida*; S. = saprozoic nematodes; c. = cysts; vc. = viable cysts; l. = larvae.

Crop 1955	Migratory nematodes							Eelworm cysts	
	P.	Pa.	M.	R.	H.	T.	O. + S.	Cereal root eelworm c. = vc. = l.	Clover and grass root eelworms c. = vc. = l.
1. Oats	90	—	—	—	35	935	2405	14 = 2 = 80	
2. Rye	**370**	—	—	—	80	990	2515	24 = 4 = 120	2 = 0 = 0
3. Rye	**420**	—	—	—	50	1250	2435	2 = 2 = 60	
4. Potatoes	70	20	—	—	50	535	1940	**70** = 24 = 680	
5. Rye	145	5	—	—	235	1360	1775	18 = 2 = 40	
6. Meadow	165	615	55	185	30	100	2545	14 = 2 = 80	2 = 2 = 20
7. Meadow	75	**455**	**85**	**470**	—	1270	2040	16 = 6 = 360	**26** = 12 = 750
8. Rye	**575**	230	5	115	—	285	2215	18 = 2 = 40	2 = 0 = 0
9. Beets	95	265	—	30	20	255	1595	18 = 2 = 40	2 = 2 = 80
10. Meadow	95	**915**	**20**	**365**	10	430	2840	24 = 2 = 40	**18** = 2 = 40

Table 1 illustrates the situation on a mixed farm with ten fields and suggests the following:

1. All plots harbour 4-6 nematode genera, which are known to include plant parasitic species. The figures show a distinct influence of the cropping history and of the lastgrown crop.

2. *Pratylenchus,* mainly *P. pratensis* (de Man) Filipjev, occurs in all plots. Its population density has evidently been promoted by rye (nrs 2, 3, 8); with potatoes and beets the densities are lower. This is in accordance with earlier data, which indicated *P. pratensis* as a parasite of cereals (Pl. XX, fig. 1), grasses, clovers and some other crops in sandy soil, whereas potatoes and beets oppressed the population (1954, 1956a).

3. All plots also harbour noticeable populations of cereal root eelworm. This is not common in the arable land of mixed farms on sandy soil, but it does reflect the intensive culture of cereals and grasses. The population on plot nr 4 obviously outnumbers that of other

fields, which is explained by the fact that oats and rye were grown here most frequently before 1955.

4. Several *Tylenchorhynchus* species are present in all fields. They live parasitically on the roots of the crop. Their significance is not fully known, but may not be very great, in spite of their dense populations.

5. Most of the arable plots harbour *Hoplolaimus uniformis* Thorne, which is known as a parasite of carrots, peas and probably of beets. The present infestation rates may be unimportant, but efficient host crops probably build up dangerous populations within a few years.

6. In the meadows (nrs 6, 7, 10) 3 undescribed *Paratylenchus* species, *Meloidogyne hapla* Chitwood, *Rotylenchus* spp. and *Heterodera* spp. on clover and grasses come to the fore (the plots 8 and 9 had also been meadows throughout 1951). *Paratylenchus* is known to include several noxious species and it is probable that the forementioned species play a role as parasites. This is also true for the *Heterodera* spp. on clover and grasses. *Meloidogyne*, here *M. hapla*, is living especially on white clover. The role of *Rotylenchus* is not known. The high populations of the four genera in the meadows nr 7 and 10, compared with the populations on adjacent plots 8 and 9 which have been arable land since 1951, indicate the salutary influence of rotation in the case of grass husbandry.

TABLE II

Influence of one year's culture of 3 different crops on the nematode population in a broken up old meadow on sandy soil.

Number of nematodes per 200 cc of soil.

T. = *Tylenchorhynchus*; P. = *Pratylenchus*; Pa. = *Paratylenchus*; R. = *Rotylenchus*; O. = other *Tylenchida*; S. = saprozoic nematodes.

Crop	T.	P.	Pa.	R.	O.	S.
Oats	**565**	**518**	253	173	163	2678
Beets	125	223	**300**	138	208	2740
Potatoes	188	443	38	**323**	160	3018
F . . . . . [1]	10,83**	5,93*	3,58*	1,91	0,57	0,16
L.S.D. at 95% level [2]	145	127	148	142	71	919
L.S.D. at 99% level [2]	217	189	222	213	106	1379

1) F = variance ratio. 2) L.S.D. = least significant difference.

The differentiating influence of the crop on the nematode complex is shown experimentally in table II. Three different arable crops were grown on four replicated plots in a newly-ploughed meadow of the

same type as indicated in table I, though situated in another sandy area. Oats have increased the *Tylenchorhynchus* and *Pratylenchus* species. The *Paratylenchus*-population is increased most by beets and the *Rotylenchus* population by potatoes.

Thus this farm harbours in each of its fields a nematode complex of 5 to 10 plant parasites. This situation has been found to be normal on farms of this kind.

It is evident that regular crop rotation, and alternate husbandry (grass/arable) prevents or restricts the development of different root eelworm populations and helps to maintain crop yields.

EFFECTS OF CROP ROTATION ON CLAY SOILS

Long-term trials showed, that on clay soils in the north of the country monoculture of cereals gave an unexplained loss of yield of about 20% in spite of good tilth and manuring (MEIJERS 1936, 1955).

TABLE III

Trial field NB on heavy clay with fixed rotations since 1934.

Number of nematodes per 800 g[1]) of soil, 1954/'55.
P. = *Pratylenchus*; Pa. = *Paratylenchus*; T. = *Tylenchorhynchus*; *O* = other *Tylenchida*; S. = saprozoic nematodes.

Permanent rotation system		Crop 1954	P.	Pa.	T.	O. + S.
SWP,	1	Sugarbeets	90	480	145	3785
	2	Wheat	**415**	**1530**	**355**	4110
	3	Peas/beans	95	560	300	6080
SWPB,	1	Sugarbeets	115	535	295	4975
	2	Wheat	**120**	**3060**	**630**	3945
	3	Peas/beans	105	640	410	4435
	4	Barley	**410**	**945**	**605**	5155
PWB,	1	Peas/beans	465	710	435	4195
	2	Wheat	**550**	**1340**	**805**	2530
	3	Barley	**405**	**1040**	**640**	4885
WB,		Wheat	**435**	**930**	**400**	2855
W,		Wheat	**725**	**740**	**725**	4370

1) For the sake of comparison a g of soil may be considered to be about equivalent to a cc of soil.

Cereal root eelworms were not present in a noticeable concentration, but soil sampling of one of these trial fields in 1954/'55 revealed a

correlation between the culture of wheat and barley and the population increase of *Pratylenchus-*, *Paratylenchus-* and *Tylenchorhynchus* species (table III). The species of this complex were different from those mentioned earlier for other soils.

Root examination in 1955 proved, that these species indeed attacked the roots of the cereals. The number of *Pratylenchus* in the roots of barley following wheat (WB) was 6 times higher than in barley following beans (SWPB, 3) in accordance with the population levels in the soil at the time of sowing. Heating of infested soil up to 60° C and fumigation with the nematicide DD gave nearly complete eelworm kill and resulted in an improvement of growth of wheat. This indicates that the eelworms caused damage to the wheat (OOSTENBRINK 1956 b). The favourable influence of rotation with beets and beans is therefore at least partly explained by the fact that they oppress the populations of these nematodes.

Table IV shows the eelworm situation in sandy clay, which was reclaimed from the sea in about 1930. From 1938 through 1952 trial strips were grown yearly with rye, wheat, barley and oats respectively; from 1953 onwards more suitable 3- and 6-year rotations were used, except on the heads of the strips where the monocultures were continued (for oats this change had already taken place in 1943).

The influence of the period of monoculture as well as the culture of the other crops is clearly reflected in the nematode populations on the different plots. The data of table IV support the following conclusions:

1. Sampling in 1954/'55 showed, that a *Paratylenchus* species (probably undescribed) has come to the fore on the "rye" strip (1-3) and a *Pratylenchus* species, which is close to or identical with *P. thornei* Sher et Allen, on the "wheat" strip (10-15). This must be a consequence of the monocultures of rye and wheat from 1938 throughout 1952. It is also shown by the heads of the strips with their continued monoculture (22 and 24). That wheat selectively increases the *Pratylenchus* is also shown by the fact, that the highest figures within each strip follow immediately after the wheat crop of 1954 (nrs 12 and 15, 5 and 9, 18).

2. Sampling in 1955/'56 confirmed the conclusions of 1954/'55. Close examination of the figures indicates that oats (nrs 1, 2, 3, 5, 20), barley (9, 23) and probably red clover (3 versus 1 and 2) also promote the *Pratylenchus*, though less effectively than wheat, whereas flax, peas, potatoes and beets seem to oppress it. The *Paratylenchus*

species seem to be promoted by rye, and also by peas (14 and 20 in 1954/'55, 9 in 1955/'56).

3. The marked increase of the *Pratylenchus* population in wheat and the marked decrease in flax or beets in 1955 illustrate the dynamic

TABLE IV

Trial field WM on sandy clay, with monocultures from 1938-'52 and other fixed rotational systems afterwards.

Number of migratory nematodes per 200 cc of soil in 1954/'55 and in 1955/'56. P. = *Pratylenchus*; Pa. = *Paratylenchus*; O. = other *Tylenchida*; S. = saprozoic nematodes; + = undersown with clover as a green manure.

Crop 1938-'52		Crop 1953	Crop 1954	Migratory nematodes 1954/'55			Crop 1955	Migratory nematodes 1955/'56		
				P.	Pa.	O. + S.		P.	Pa.	O. + S.
Rye,	1	wheat	barley	3	**2255**	866	oats	70	740	1560
	2	wheat +	barley	0	**920**	1659	oats	10	750	1390
	3	wheat +	barley +	0	**2073**	1751	oats	220	480	1300
Barley,	4	oats	flax +	3	25	1069	wheat	20	200	720
	5	flax +	wheat	**73**	30	2181	oats	260	90	1340
	6	wheat	oats	0	65	1575	flax +	0	60	910
	7	wheat	barley +	20	168	4713	beets	10	80	700
	8	barley +	beets	13	248	2066	wheat	200	190	1210
	9	beets	wheat	**90**	785	1191	barley +	320	660	1320
Wheat,	10	wheat	flax +	**210**	168	2586	potatoes	210	40	890
	11	flax +	potatoes	**375**	50	2395	wheat	**1570**	80	780
	12	potatoes	wheat	**1388**	140	2127	flax +	**390**	130	940
	13	wheat +	beets	**355**	138	1121	peas	120	120	680
	14	beets	peas	**143**	545	1346	wheat +	**580**	420	890
	15	peas	wheat	**420**	203	3723	beets	**130**	110	750
Oats [1]),	16	oats	flax +	15	63	2268	potatoes	40	0	1250
	17	flax	potatoes	68	33	966	wheat +	**400**	220	650
	18	potatoes	wheat +	**185**	243	1588	beets	**90**	60	940
	19	wheat +	beets	93	243	2018	peas	30	470	970
	20	beets	peas	5	713	641	oats	20	200	600
	21	peas	oats	5	100	1158	flax +	30	70	1380
Rye,	22	rye	rye	25	**618**	526	rye	20	**1610**	1300
Barley,	23	barley	barley	40	58	963	barley	110	110	1560
Wheat,	24	wheat	wheat	**270**	123	1078	wheat	**370**	410	1030
Oats [1]),	25	[1])	[1])	8	188	2098	[1])	30	300	700

1) On the oats strip oats were grown from 1938-'43 only and flax/peas from 1944-'55 to avoid damage by the cereal root eelworm.

character of the population. In comparing 11 versus 12, 14 versus 15, 17 versus 18, the infestation level varies by a factor of 15, 16 and 13 times respectively.

Fumigation with DD or heating for 2 hours at 60° C eliminated a marked growth inhibiting factor in rye in soil from plot nr 22 (Pl. XX, fig. 2) and in wheat in soil from plot nr 11. This suggests that the nematodes, probably the corresponding *Paratylenchus* and *Pratylenchus* species, cause damage. Part of the damage could be caused by the cereal root eelworm of which a very slight infestation of 0.2 and 0.7 larvae per cc of soil was present, but rye and wheat are not likely to suffer visibly from such a light infestation. Besides this, *Pratylenchus thornei* is also encountered as the cause of growth stagnation in barley and in apple trees in other clay soil areas.

Thus it seems that in this soil, at least 3 different nematode problems exist, in close connection with the cropping history and that only systematic crop rotation will be able to prevent damage. This has already been stressed for cereal root eelworm in the same soil (Dijkema 1947).

THE DESIRABILITY OF CROP ROTATION ON NEWLY-RECLAIMED SOIL

In newly-reclaimed polder soil it is normally possible to grow good crops for a number of years, without rotation (Meijers 1955). Plant

TABLE V

Number of nematodes of different genera per 200 cc of newly-reclaimed polder soil, in comparison with old cultivated soil round about.

P. = *Pratylenchus*; T. = *Tylenchorhynchus*; H = *Hoplolaimus*; R. = *Rotylenchus*; O. = other *Tylenchida*; S. = saprozoic nematodes.

	P.	T.	H. + R.	O. + S.
New soil				
Parcel D 14 (3rd crop)	—	—	—	4215
Parcel D 115 (4th crop)	—	85	—	5060
Parcel D 127 (3rd crop)	—	10	—	4130
Parcel D 126 (3rd crop)	—	60	40	6060
Old soil				
Vollenhove (arable land)	30	3970	30	3760
De Voorst (arable land)	30	3430	120	7860
Urk (turf)	100	4600	1100	8890
Kampen (turf)	900	1800	1300	27200
Lemmer (turf)	1600	2300	1000	29200

parasitic nematodes are virtually absent. They were also not detected in appreciable numbers by the examination of soil or crop samples in a number of fields up to the fourth crop on a new sea polder. This was markedly different from older cultivated land round about (table V). In monocultures, however, and also in certain cropping sequences, occurrence and increase of certain *Pratylenchus* and *Paratylenchus* species takes place within a few years.

Table VI shows the eelworm situation in newly-reclaimed sandy clay, where immediately after reclamation in 1944 a trial was started with rye, wheat, barley and oats, each crop being grown as follows: a) monoculture, b) two crops in three years, c) one crop in three years. Plots bearing treatments (b) and (c) were split between 6 different crops in the non-cereal years.

In 1955, the 12th year, cereals were grown in the whole field, i.e. rye in the rye strip, wheat in the wheat strip etc. In this year slight differences in growth appeared, which were connected with the cropping history and possibly due to eelworm damage (GROOTENHUIS 1956).

In 1955 root samples and soil samples from 32 plots were examined. *Heterodera* species were not observed. The following conclusions can be drawn from table VI:

1. During the 12 years of cultivation species of *Pratylenchus* and *Paratylenchus,* different from those mentioned earlier, have become established at various levels.

2. *Pratylenchus* seems to be promoted most by wheat, less by oats and barley and least by rye (nrs 1, 9, 17, 25 and 2, 10, 18, 26). The high numbers in wheat following beets or potatoes in 1954 (nrs 11 and 12), and the very high numbers in wheat following beets or potatoes in 1953 and cereals in 1954 (nrs 13 and 14) indicate, that beets and potatoes are even more efficient host crop than the cereals. The corresponding fields in the other strips indicate the same. Rotation with rye, flax and peas and probably also with beans, seems to oppress the *Pratylenchus* population.

3. The *Paratylenchus* species seems to have risen primarily in the plots where potatoes and beets have been followed by 2 years of cereals (nrs 5, 13, 14, 29).

Fumigation of a moderately infested strip (about 400 *Pratylenchus* and 20 *Paratylenchus* per 200 cc) with DD killed about 75 % of the *Pratylenchus* and gave growth improvement in barley, potatoes and some grasses, but not in several other crops.

A similar situation exists in newly reclaimed soil in peat regions; originally no root eelworms are present but after some years of

TABLE VI

Trial field NOP on newly-reclaimed sandy clay, with fixed rotational systems since 1944.

Number of nematodes per 10 g of roots in May 1955 and per 200 cc of soil in December 1955.

P. = *Pratylenchus*; Pa. = *Paratylenchus*; O. = other *Tylenchida*; S. = saprozoic nematodes.

Permanent rotation system		Crop 1953	Crop 1954	Crop 1955	Per 10 g of roots May 1955		Per 200 cc of soil December 1955		
					P.	O.+S.	P.	Pa.	O.+S.
Rye	1	Rye	Rye	Rye	85	270	160	110	1000
2/3 Rye	2	6 crops	Rye	Rye	0	730	0	80	1095
1/3 Rye	3	Flax	Potatoes	Rye	5	235	0	10	1430
	4	Beans	Beets	Rye	20	525	20	125	2265
	5	Beets	Oats	Rye	1155	1050	50	520	1625
	6	Potatoes	Wheat	Rye	20	265	45	135	1320
	7	Wheat	Peas	Rye	0	1020	55	295	1275
	8	Oats	Flax	Rye	25	115	40	165	1310
Wheat	9	Wheat	Wheat	Wheat	3790	730	1435	85	1190
2/3 Wheat	10	6 crops	Wheat	Wheat	3410	375	1445	112	939
1/3 Wheat	11	Flax	Potatoes	Wheat	2975	170	1075	40	1145
	12	Beans	Beets	Wheat	2175	275	1210	115	1220
	13	Beets	Oats	Wheat	2860	405	1470	2020	920
	14	Potatoes	Barley	Wheat	6590	270	1405	1090	560
	15	Barley	Peas	Wheat	105	940	353	340	920
	16	Oats	Flax	Wheat	60	285	360	105	875
Barley	17	Barley	Barley	Barley	961	311	160	80	740
2/3 Barley	18	6 crops	Barley	Barley	750	175	445	395	935
1/3 Barley	19	Flax	Potatoes	Barley	344	232	325	25	1635
	20	Beans	Beets	Barley	645	105	205	205	1300
	21	Beets	Oats	Barley	6555	230	1380	107	1148
	22	Potatoes	Wheat	Barley	4861	417	955	85	680
	23	Wheat	Peas	Barley	35	415	95	0	570
	24	Oats	Flax	Barley	206	131	55	5	805
Oats	25	Oats	Oats	Oats	2775	355	250	5	960
2/3 Oats	26	6 crops	Oats	Oats	1745	455	175	30	920
1/3 Oats	27	Flax	Potatoes	Oats	285	360	100	115	1215
	28	Beans	Beets	Oats	130	1160	175	20	1025
	29	Beets	Barley	Oats	520	320	150	1230	615
	30	Potatoes	Wheat	Oats	3290	295	605	125	780
	31	Wheat	Peas	Oats	65	820	35	55	855
	32	Barley	Flax	Oats	5	965	15	5	750

Fig. 1. Growth inhibition in rye on diluvial sandy soil with a high population of *Pratylenchus pratensis* and *Tylenchorhynchus* spp. Left, after treatment of the soil with the nematicide DD.

Fig. 2. Growth inhibition in rye in sandy clay soil with a dense population of an undescribed *Paratylenchus* species (plot nr 22 of table 4). Fumigation of the soil with the nematicide DD (centre) and heating the soil to 60° C (at left) removed the nematode factor and increased the number of ears 2½-3 fold.

cultivation *Pratylenchus* and other parasitic species occur and are correlated with decrease of crop yields.

Crop rotation in newly reclaimed land is therefore at least partly ruled and imposed by natural invasion and increase of *Pratylenchus, Paratylenchus* and other root eelworm species. Rotations should be planned so as to minimise increase, but more work is required before this can be done with confidence.

NEMATODE PROBLEMS IN NURSERIES AND PRIVATE GARDENS IN CONNECTION WITH THE SEQUENCE OF CROPPING

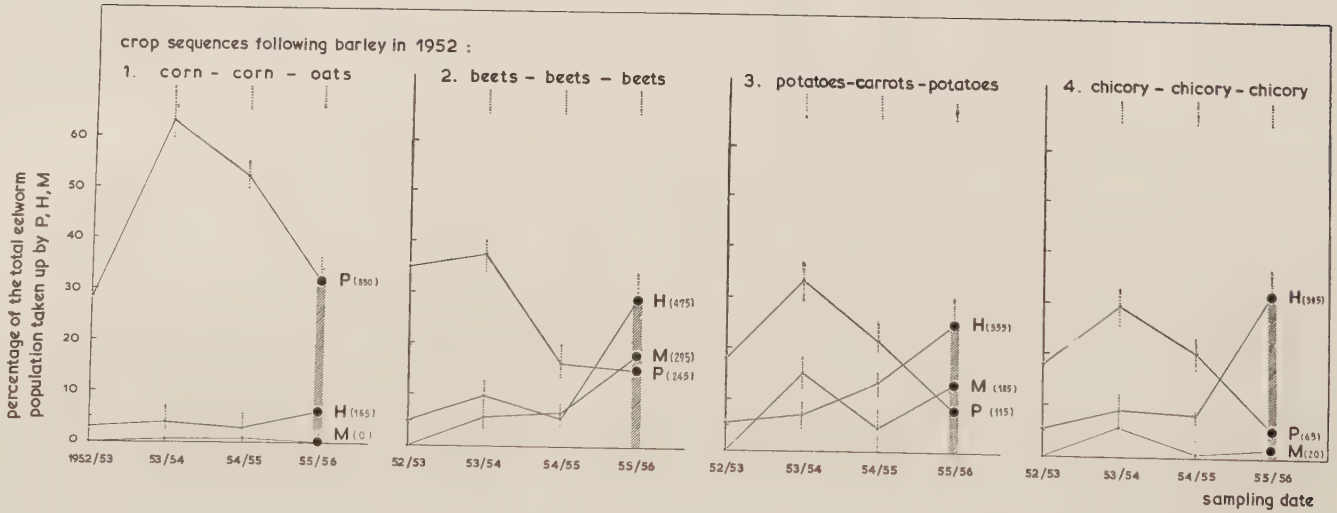
In nurseries and gardens, apart from *Heterodera* infestations, other nematode problems may be encountered than in surrounding arable land. Often *Meloidogyne hapla* and *Hoplolaimus uniformis* or *Pratylenchus penetrans* and *Paratylenchus* species are important (1954). Here also the sequence of crops often appears as the ruling factor.

Fig. 1 demonstrates the influence of change of arable farming to horticulture (no cereals), upon the characteristic eelworm problems for sandy soil. In an arable field, where barley was damaged by *Pratylenchus pratensis* in 1952, *P. pratensis* was present at a high, and *Hoplolaimus uniformis* and *Meloidogyne hapla* at a low level in 1952/'53.

Certain crop sequences, practised in adjoining plots for a number of years, however, changed the eelworm pattern markedly. The relatives of the different eelworm species are given in fig. 1. After three years *H. uniformis* and *M. hapla* were prevalent in the sequences "beets-beets-beets" and "potatoes-carrots-potatoes". The sequence "chicory-chicory-chicory" promoted *H. uniformis,* but evidently oppressed the two other species. Only in the sequence "corn-corn-oats" was the original situation maintained with *P. pratensis* at a high and the other species at a low level.

The levels at the last sampling date are placed between brackets in the graphs. The *Meloidogyne* levels in the sequences 2 and 3 and the *Hoplolaimus* levels in the sequences 2, 3 and 4 are considered to be sufficiently high to cause damage to a number of vegetable and ornamental crops. The *Pratylenchus* level in sequence 1 may be noxious to lawn grasses and some other crops. Thus after three years quite distinct eelworm problems have built up within a distance of some metres under the different crops.

Fig. 1. Increase of certain components of a common eelworm spectrum in sandy soil by different cropping sequences. Nematodepopulations between brackets, per 200 cc of soil. P = *Pratylenchus*; H = *Hoplolaimus*; M = *Meloidogyne* larvae.



CONCLUSION

The foregoing data indicate the presence of a dynamic, noxious nematode population in nearly all cultivated soil. The examples given are considered representative, since any trial field or any farm so far examined shows a similar complex of root-attacking eelworms in all plots, with only newly reclaimed or artificially disinfected soil as an exception.

There appears to be a close connection between the cropping sequence and the composition of and damage by the nematode complex. It cannot be predicted, however, what proportion of hitherto unexplained crop rotation effects is generally due to nematodes, since other plant diseases and fertility factors also play a part (c.f. OOSTENBRINK 1956 a).

The authors gratefully acknowledge all those who helped in this work. Several colleagues and assistants of the Plantenziektenkundige Dienst participated in the technical work. We are indebted to the officers of the „Rijkslandbouwvoorlichtingsdienst" and „Toegepast Natuurwetenschappelijk Onderzoek" for allowing us to study the eelworm situation in their rotation trials. Mr F. G. W. JONES gave much help and advice in preparing the English text.

ZUSAMMENFASSUNG

Eine Erklärung für einige Fruchtwechselerfahrungen, begründet auf Aelchen-Beobachtungen und Populations-Studien.

Die vorliegende Arbeit illustriert das allgemeine Auftreten eines dynamischen, multiplen Wurzelälchenfaktors in Kulturböden und die Bedeutung der Nematoden als wuchshemmender Faktor in Verbindung mit der Fruchtfolge. Die Daten beziehen sich hauptsächlich auf holländische Böden; Untersuchungen im Ausland weisen auf die internationale Bedeutung dieses Problems.

Kultivierter Boden beherbergt normal, abgesehen von *Heterodera*-Arten und saprozoischen Formen, einen Komplex verschiedener parasitischer Wurzelälchen, wie *Pratylenchus-, Paratylenchus-, Rotylenchus-, Tylenchorhynchus-, Hoplolaimus-* und *Meloidogyne*-Arten.

Tabelle 1 gibt den Stand auf einem gemischten Sand-Bauerngut wieder und zeigt in jedem Felde die Anwesenheit von 4-6 Gattungen, bzw. 5-10 Arten von parasitischen Wurzelälchen auf. Die Ziffern zeigen einen entschiedenen Einfluss des Fruchtwechsels und des letzten Gewächses. Der differenzierende Einfluss des Gewächses wird experimentell in Tabelle II wiedergegeben. Verschiedene Arten in den Tabellen I und II sind bekannte Pflanzenparasiten, und von anderen wird angenommen, dass sie gewisse Gewächse schädigen (Pl. XX, Fig. 1). Es ist deutlich, dass Fruchtwechsel, wie auch der Wechsel zwischen Weide- und Feldwirtschaft in der Praxis dazu beiträgt, dass die Ernten auf der Höhe bleiben.

Auf Tonböden trifft man in der Praxis dieselbe Sachlage an, obwohl hier andere Aelchenarten eine Rolle spielen.
Tabelle III zeigt die Beziehung zwischen Weizen- und Gerste-Kultur und den Anwuchs der Population von *Pratylenchus-*, *Paratylenchus-* und *Tylenchorhynchus*-Arten in einem Versuchsfelde auf schwerem Tonboden.
Tabelle IV zeigt die Situation in einem beständigen Fruchtwechselversuchsfelde auf sandigen Lehm. Spezifische *Pratylenchus-* und *Paratylenchus*-Arten treten bei gewissen Gewächsen in den Vordergrund und werden von anderen unterdrückt. Die starke Entwicklung der *Pratylenchus*-Population in Weizen und die starke Abnahme derselben in Flachs und Rüben im Jahre 1955, illustrieren den dynamischen Charakter der Population. In beiden Feldern zeigt Ertragsverlust in der verseuchten Erde, und Zurückkehr der Fruchtbarkeit nach einer Bodenbehandlung mit einem Nematizide (DD) oder mit Hitze (60° C), die Bedeutung des Nematodenfaktors (Pl. XX, Fig. 2).
Der günstige Einfluss der Fruchtwechsels ist hier also mindestens teilweise durch die Tatsache erklärt, dass die Aelchen-Populationen hierdurch in Schranken gehalten werden.

Auf jungfräulichen Böden ist es gewöhnlich möglich, gute und gleichmässige Gewächse während einer Reihe von Jahren ohne Fruchtwechsel zu erzielen. Pflanzenparasitische Aelchen sind nicht oder kaum anwesend (Tabelle V).
Tabelle VI dagegen illustriert, dass innerhalb von 12 Jahren nach der Bebauung spezifische *Pratylenchus-* und *Paratylenchus*-Arten eingedrungen sind und in einigen Kulturen und bei gewissen Fruchtfolgen eine hohe Population aufgebaut haben. Es ist wahrscheinlich, dass die Notwendigkeit des Fruchtwechsels in neu bebauten Gelände wenigstens teilweise von diesem Nematodenfaktor geregelt und geleitet wird.

In Züchtereien und in Privatgärten werden oft andere Aelchen-Probleme angetroffen als im angrenzenden Ackerland. Fig. 1 demonstriert eine schnelle Veränderung von Ackerland in Gartenwirtschaft (kein Getreide), so weit es einige charakterische Aelchenprobleme auf Sandboden betrifft. Eine dreijährige Umstellung der Fruchtfolge genügte, um *Pratylenchus pratensis* zu unterdrücken und *Meloidogyne hapla* und *Hoplolaimus uniformis* in die Höhe zu bringen.
Auch im Gartenbau ist es also logisch anzunehmen, dass die Fruchtfolge für das Auftreten der verschiedenen Aelchen-Probleme bestimmend ist, und dass die Fruchtfolge von Jahr zu Jahr die Probleme beeinflusst.

Die Gegenwart eines polyfaktoriellen Aelchenkomplexes in kultiviertem Lande und der Einfluss der Fruchtfolge auf den Aelchenbestand werden als allgemeine Phänomene betrachtet. Alle Versuchsfelder und Farmen, die zufällig auf Nematoden geprüft wurden, bestätigen diese Auffassung. Es war bekannt, dass Fruchtwechselwirkungen in speziellen Fällen eine Folge von Aelchengradationen sind. Die heutigen Befunde aber stützen die Auffassung, dass der Fruchtwechselmechanismus sehr allgemein mit dem Aelchenfaktor verknüpft ist. Gewisse Beobachtungen haben die Autoren von der Wichtigkeit dieses Faktors überzeugt. Es kann jedoch nicht mit Sicherheit ausgesagt werden, in wie weit nicht geklärte Fruchtwechselergebnisse im allgemeinen dem Nematodenbefall zugeschrieben werden können, da hierbei auch andere Faktoren eine Rolle spielen (siehe OOSTENBRINK 1956a).

Die Befunde enthüllen auch im Anschluss an frühere Berichte (1954) den Einfluss des Bodentypus auf den Wurzelälchenkomplex. Es ergibt sich, dass der Bodentypus hoch selektiv oder sogar bestimmend ist für das Auftreten gewisser *Pratylenchus-* und *Paratylenchus*-Arten. Dies scheint nicht dermassen zu gelten für *Hoplolaimus uniformis* und *Heterodera*-Arten.

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DISCUSSION

J. VAN DEN BRANDE: commented upon the perspectives for further work and stressed the value of international contacts in this field.

J. H. SCHUURMANS STEKHOVEN: How does infestation with plant parasitic nematodes take place in newly-reclaimed poldersoil.

ANSWER: Transport of soil-adhering products from the old land and into or across the polders probably results in infestation along the roads. New soil does not harbour plant parasitic nematodes, but after some years of cultivation certain species come to the fore, even in fields where no import by seed, manure or implements can be traced or expected. Wind and wild animals or birds may also play a role.

W. J. VAN DER LINDE: When in your trials the *Meloidogyne* species attacks corn, it is probably not *M. hapla* but *M. arenaria thamesi.*

ANSWER: This species is not favoured but oppressed by corn, as is shown in fig. 1.

H. R. WALLACE: What is your opinion of the generalisation that heavier infestations of *Ditylenchus* occur in clay soils, and that in the case of cyst-forming species heavier infestations occur in the sandy soils.

ANSWER: Could it be that *Ditylenchus* is more dependable on wet soil since it has to move towards the stem of the plants whereas the locomotion of *Heterodera* larvae can be limited as they are "visited" by the roots?

O. AHLBERG: remarked that in Sweden potato root eelworm does not reproduce in sandy soils as rapidly as in other soils. The infestation in sandy soils, however, is more serious, because potatoes are mainly grown in sandy districts.

END OF SYMPOSIUM PAPERS.

(*Nematologica*, Vol. I, 1956, no. 3)

NEW TECHNIQUES FOR COUNTING AND ISOLATING FREE LIVING NEMATODES FROM SMALL SOIL SAMPLES AND FROM OAK FOREST LITTER *)

BY

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In an article on aims and methods of ecological research on nematodes (MINDERMAN, 1956) five techniques are mentioned. Four of these methods are described below. A description of the fifth, the preparation of sections of soil samples suitable for microscopical investigation at high magnifications, will be published elsewhere. The methods described here are:

1. Two techniques for quantitative investigations of eelworms in small soil samples.
   a. a staining method.
   b. a centrifugal flotation method.
2. Two techniques for quantitative investigations of nematodes in forest litter.
   a. a modification of the Baermann funnel method.
   b. a bleaching and staining technique.

QUANTITATIVE INVESTIGATIONS OF EELWORMS IN SMALL SOIL SAMPLES

a. *The staining method.* For investigations on the ecology of soil inhabiting nematodes reliable quantitative techniques are needed for the determination of the numbers of all species of nematodes present in small samples, ¼ ml to 1 ml in size. The technique must be applicable to different types of soil including peat. None of the methods described in literature was found to be wholly satisfactory. The best results were obtained when using the direct examination technique described by STÖCKLI (1943). This is a time consuming method, however, which is also very tiring to the eyes. A great improvement was obtained by combining it with the lactophenol cottonblue staining of the nematodes as described by GOODEY (1937), for the demonstration of nematodes in plant tissue. In this way the nematodes

*) Received for publication: September 15, 1956.

in the soil sample are stained deep blue, whereas the mineral and organic soil particles remain unstained. The nematodes are then readily visible between these particles and it is much easier to count and to collect the smallest specimens under low power (50 ×) than when STÖCKLI's technique is used.

Procedure

1. A soil sample of ¼ of ½ ml. is placed on filter paper in a filter holder as shown in Pl. XXI.

2. A solution of 0,05 % cotton blue or acid-fuchsin in lactophenol is heated to about 70° C and poured on the sample.

3. After 3-5 minutes the nematodes have absorbed sufficient stain and the lactophenol cotton blue is sucked from the soil sample through the filter paper by means of a suction pump. Some plain lactophenol is poured on afterwards and also sucked through in order to remove all excessive stain.

4. A small amount of lactophenol is added to the sample which is then transferred to a counting dish using the tip of a feather as a brush.

5. Under a dissecting microscope (50 ×) the nematodes are picked from between the soil particles and transferred from the counting dish to drops of lactophenol on slides by means of a hair fixed to a glass rod. The drops are covered afterwards by cover slips, which are sealed with Thorne's Zut (Glyceel).

The filterpaper used is Delta No 350, a hard, smooth and tough quality, which is commonly used for filtering strong acids or alkaline solutions. It does not give off loose fibres.

In the identification of the nematodes mounted in lactophenol the phase contrast microscope is a valuable help.

b. *The centrifugal flotation method*

The staining technique is very valuable as a method for checking the relability of other techniques but it takes to much time to be used as a routine method for the investigation of large numbers of samples. The centrifugal flotation method described below requires much less time and is therefore more appropriate for this purpose. The technique proved to be applicable to samples of sizes between ¼ ml and 4 ml, depending on the humus content of the soil.

Sandy soils and clay soils are treated in different ways.

Equipment:

1. A centrifuge with tubes of 50 ml and 100 ml and fitted with a control to permit the number of revolution per minute to be regulated. A centrifuge with 15 ml tubes, driven by hand, may also meet the requirements of the method in a number of cases.

2. An electric stirrer, with a propeller small enough to operate in 50 ml centrifuge tubes.

3. A solution of magnesiumsulphate in water, spec. gravity 1.25.

4. Filter holders and filter paper as described above.

5. Counting dishes, preferably with sloping sides.

Procedure.

I. For sandy soils.

1. The sample is transferred to a 50 ml. centrifuge tube which is filled with magnesiumsulphate solution up to ¼ or ½ of its volume. This tube is clamped under the stirrer, which is run fast enough to suspend the particles of the soil sample in the solution. This stirring process takes five minutes.

2. The tube is taken from the stirrer, some more mag. sulph. solution is added until the tube is nearly filled and then placed in the centrifuge for one minute. After about 30 seconds the number of revolutions must be constant at about 1500 revolutions per minute. It is stressed here that centrifuges may differ in the time needed to reach top speed and the figures given here may be taken as a guide but will vary with other machines.

3. After centrifuging, the clear supernatant part of the liquid is poured into a filterholder, taking care that the tube is rotated on its longitudinal axis to ensure that the parts adhering to the wall of the tube also come on to the filter. This part of the liquid is then sucked through by means of a pump.

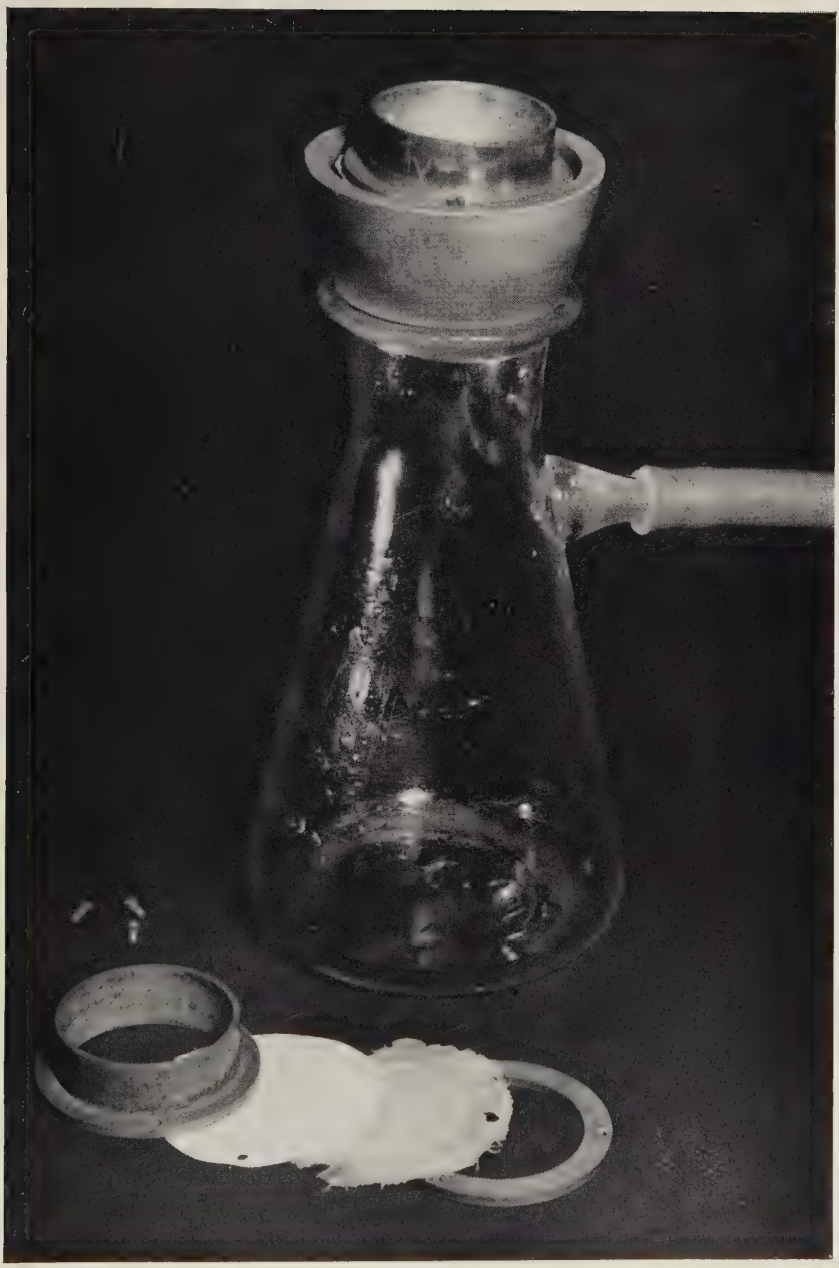
4. More mag. sulph. solution is added to the heavy portion which remains in the tube. This is now shaken or stirred for a short time and is followed by centrifuging as in paragraph 2 above.

5. As paragraph 3, using the same filter.

6. The residue on the filter is washed with water and then stained by pouring over it a hot solution of 0.05 % acid fuchsin or cotton blue in lactophenol.

7. After five minutes the dye is sucked through and the residue washed with plain lactophenol.

8. The residue is transferred from the filter into a counting dish.



Filterholder on a vacuum flask. At the bottom from left to right the parts of this holder: ring with flange, filter paper, piece of cotton, second ring.

The shaking apparatus assembled. (See fig. 1).

The nematodes are then picked from the residue and placed on microscopic slides with the help of a hair and a binoculair low-power microscope (50 ×).

II. For clay soil.

1. The sample is transferred to a 50 ml centrifuge tube which is filled up to $^1/_4$ or to $^1/_3$ of its volume with the mag. sulph. solution. This tube is clamped under the stirrer, which must revolve at sufficient speed to suspend the particles of the soil sample in the solution. Stirring takes five minutes.

2. The tube is taken from the stirrer, a new quantity of the salt solution is added until the tube is nearly filled and the tube is then placed in the centrifuge for one minute. After about 30 seconds the number of revolutions must be constant at about 1500 revolutions per minute.

3. After centrifuging, the supernatant fluid is poured into a 100 ml centrifuge tube, taking care that the 50 ml tube is rotated on its longitudinal axis to ensure that the fluid adhering to the wall also goes into the 100 ml tube.

4. More mag. sulph. solution is added to the heavy portion which remains in the 50 ml tube, which is now shaken or stirred for a short while followed by centrifuging as in paragraph 2 above.

5. As paragraph 3, using the same 100 ml tube.

6. The 100 ml tube is placed in the centrifuge and rotated for five minutes at a rate of 2500 r/m.

7. After centrifuging, the clear supernatant part of the liquid is poured into a filterholder, taking care that the tube is rotated on its longitudinal axis.

8. More mag. sulph. solution is added to the heavy portion remaining in the tube, which is now shaken or stirred for a short while followed by centrifuging as in paragraph 2 above.

9. As paragraph 7, using the same filter.

10. Washing, staining etc.

Numbers of nematodes extracted from samples of different soils by the centrifugal flotation method and found in the residues by the staining method are given in table I.

TABLE I

Numbers of nematodes extracted from different soils by centrifugal flotation. Sample size: ¼ ml.

Topsoil (uppermost 3 cm) from:	Number of nematodes found on the filter	Number of nematodes found in the residues in the centrifuging tubes [1]).
Meadow on sand	116	1
Meadow on sand	189	1
Dry heath on very poor sand	16	2
Dry heath on very poor sand	9	0
Dry heath on very poor sand	34	0
Oak forest on heavy clay over peat	59	1
	423	5

1) Determined by the staining method, using Acid Fuchsin in lactophenol.

COUNTING AND EXTRACTING NEMATODES FROM OAK FOREST LITTER

a. *Extraction by intermittent shaking (Modification of the Baermann funnel method)*

Besides the number of nematodes in soil, the author wished to determine the number present in oak forest litter, i.e. in leaves in all stadia of humification. The simplest method for the separation of nematodes from such material is the well known Baermann-funnel-technique. The results obtained by this method were, however, not promising. Many days elapsed before nematodes ceased to collect in the stem of the funnel. Also, a number of nematodes probably stayed between the leaves and the presence of an unknown number of eggs gave rise to a new juvenile population. To overcome these difficulties a modification of this technique was designed introducing intermittant shaking.

Procedure.

About 10 g of the material to be examined is cut into pieces of about 1 sq. cm. From this well-mixed material a small sample of about 300 mg is placed on a piece of copper gauze fastened about 2 cm from the bottom in a 100 ml beaker (see fig. 1). This beaker is filled with water op to $^1/_2$ or $^2/_3$ of its volume. The beaker replaces the funnel of the Baermann technique. One to six beakers are placed in the carrier of the apparatus of fig. 1 and Pl. XXII and are shaken periodically for 24 hrs. Each period of shaking lasts 1 minute, followed by 4 minutes of rest.

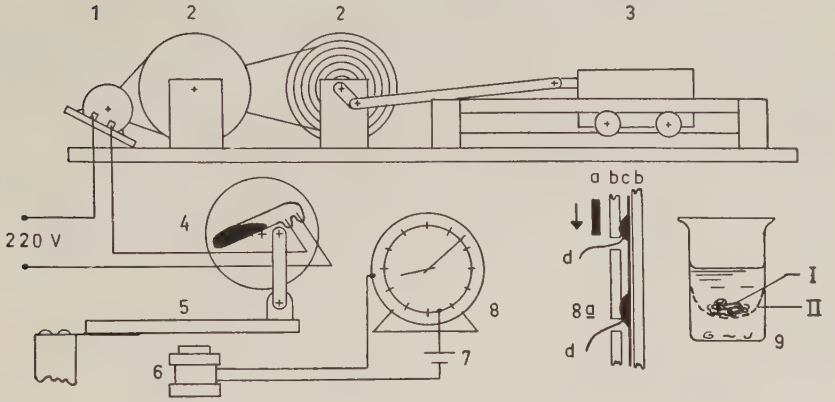


Fig. 1. Arrangement of the apparatus in use: 1. Selfstarting motor, 20 Watt, 220 Volt, 1400 r/m. 2. Stepped pulley, wood, diameter 15 cm., 6 steps. 3. Shaker-carrier moving between two pair of rails. 4. Mercury-switch mounted on a vertical turntable, connected by a lever with the soft-iron bar (5) mounted on a leafspring. 6. electrical magnet connected with a time switch clock (8) via a battery of 4 V. (7). 9. 100 ml. beaker partly filled with water. The sample (I) rests on a copper gauze (II). The time switch is a modified alarm clock. The dial-plate is replaced by 2 pieces of plastic, (b) between them a ring of copper-wire (c) on which are soldered 12 contact springs (d) which pierce through the uppermost plastic layer and make periodic contact with the minute-hand (d.)

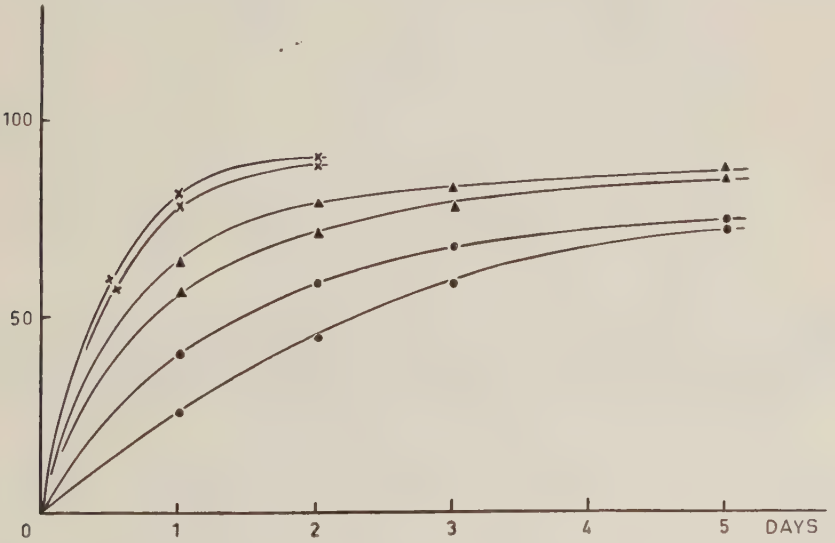


Fig. 2. Results obtained by different techniques. On the ordinate the number of nematodes caught, expressed in % of the estimated number originally present in the sample.

—●— Baermann-technique.
—▲— Intermittent shaking, 2 periods of 5 minutes per hour.
—x— Intermittent shaking, 12 periods of 1 minute per hour.

The amplitude of movement of the carrier is 2.5 cm. and one movement is completed in 3 seconds. If at the end of the shaking period the bottom of the beaker is found to be covered by too much fine material to find the nematodes easily, the whole layer is transferred to a sieve (aperture 50 μ) which just touches the water surface in a

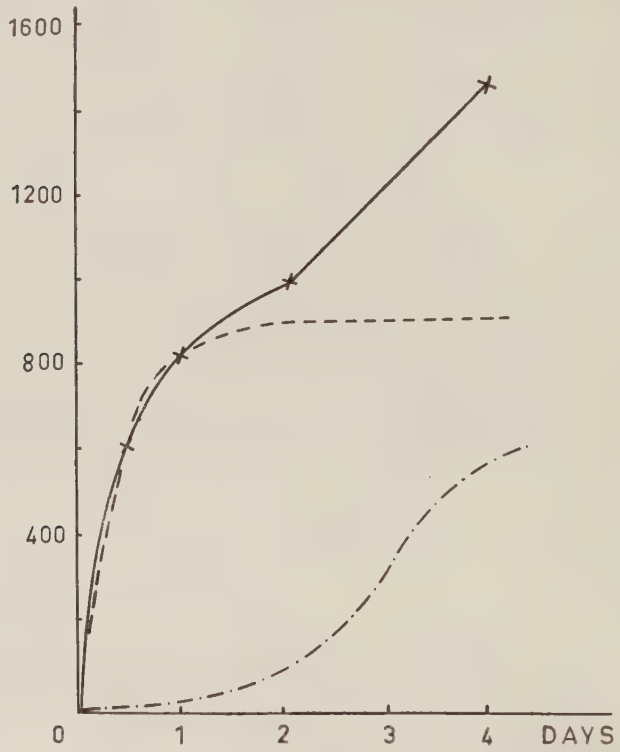


Fig. 3. Diagram to show the derivation of the curve representing the estimated number of newly hatched larvae (—.—) from the curve observed (————) and the hypothetical exponential curve (- - -) with its asymptotic value = 1. Ordinates, the number of nematodes caught in sample D, weight 1050 mg dry litter, taken from the same parent material as the samples A, B, C in fig. 1.

Petri-dish. After an hour practically all nematodes will be found in the clean dish.

This method appeared to be the best of those tested methods. Use of the Waring Blendor to cut the leaves into very small particles resulted in halving about 30 % of the nematodes. It was found that by shaking for 5 minutes every half-hour, more nematodes were extracted from samples of the same material than by the Baerman

funnel method and less than by more frequent shaking (fig. 2). For this reason the technique outlined above was the most satisfactory and it was therefore analysed more closely.

Using Baermann funnels, moss samples (*Pleurozium spec.*) yielded 50 % of the number of nematodes originally present after 2 hours and 96 % after 18 hours. The extraction curve approximated closely to that of the exponential function $Y = 1-(\frac{1}{2})^x$. Extraction of nema-

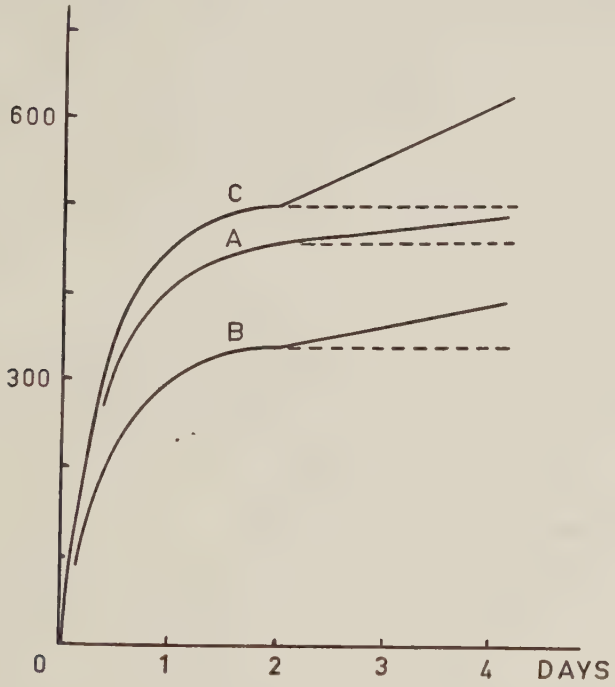


Fig. 4. Three diagrams showing the departure of the observed curve from the hypothetical (- - - - -) exponential curve. Ordinates, the number of nematodes in three samples which were taken from the same parent material. Weights of the samples A, B and C resp. 305, 288 and 256 mg. dry litter respectively.

todes was completed in this case within 24 hours because the moss had no cavities in which the eelworms could stay. The extraction curve for soil samples using the Baermann funnel method was also found to approximate to an exponential function.

In experiments with litter, however, a departure from the exponential curve appeared after 48 hours of extraction (fig. 3, 4). The author suggests that the extraction curve for nematodes from any

sample may be represented by the exponential function $Y = 1 - (\frac{1}{2})^x$. If this assumption is correct the curves found for litter may be split into:

a. the hypothetical exponential curve

b. a second curve superimposed over the exponential one and thought to represent the number of newly hatched larvae.

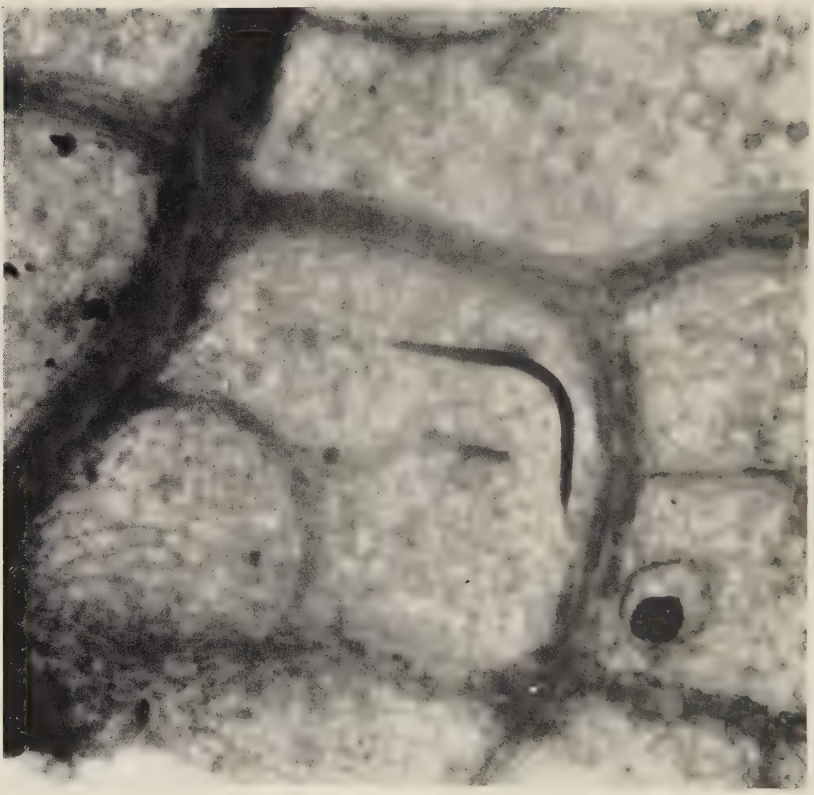
In the example shown in fig. 3 only 1 adult female occurred in the period between 48 and 96 hours among 498 nematodes found; all others were larvae. Between 24 and 48 hrs the percentage of adults was about 50 %. The second curve suggests that in the beginning only a small number of newly hatched larvae disturb the true numbers.

As all the nematodes are not shaken out and collected, the exponential curve obtained is only an approximation of the number originally present. To this number must be added that of nematodes still present in the litter after 96 hrs shaking (for the technique of finding these eelworms see p. 220). In example A (fig. 4) 489 eelworms were collected after 96 hrs. and 49 eelworms were found still present in the treated litter. An approximation of the number of newly hatched larvae from the superimposed curve gave 26. The number originally present should have been $489 + 49 - 26 = 512$ of which $489\text{-}26 = 463$ i.e. 89 % were collected.

Litter from another situation produced by other plants would give a different percentage of extraction rates.

From the diagrams it appears that after 24 hrs shaking the disturbance due to newly hatched larvae is very small and also that the percentage of the original population collected is rather constant, being 80-82% in our samples of oak forest litter. This percentage was accepted as constant and the data obtained after 24 hours shaking were multiplied by the factor 1.25 to approximate as closely as possible to the number of nematodes originally present in the sample.

The results of 3 tests of the Baermann-technique using samples of the same material, showed that 60 nematodes per 100 mg litter were found using the latter and 157 eelworms per 100 mg. using the shaking technique. The shaking technique is still somewhat selective, as the species living in the leaves are extracted proportionally less readily than the species living between the leaves. It is possible to identify and to count the numbers still present in the leaves by bleaching and staining them and teasing out the nematodes by means of needles under a low-power binoculair microscope.



Nematode in an oak leaf from forest litter.

b. *Direct microscopical examination of the organisms present in dead oak leaves.*

After removing most of the nematodes from oak litter the number still present in the leaves had to be determined. The brown opaque leaves were bleached with hydrogenperoxyde solution and were made transparent by immersing in lactophenol. Nematodes and other organisms (fungi and baceteria) present were stained by means of stains dissolved in lactophenol.

Damp leaves or pieces of them are covered with a mixture containing approximately 3 vol. water, 1 vol. 20 % ammonia sol., 1 vol. 30 % hydrogenperoxyde (JOHANSEN, 1940).

The bleaching process takes 1 to 24 hours, dependent on the nature of the material to be bleached. After 24 hrs in the bleaching agent the nematodes can still be satisfactorily stained (Pl. XXIII). The bleached litter is first washed in water and then stained by pouring the hot stain solution (± 65° C) over the leaves. After about 5 minutes the dye is poured off and is replaced by pure lactophenol until no appreciable amount of dye enters into the washing fluid. The objects are mounted in lactophenol and sealed by "Zut".

Stains suitable for our purpose were: Trypanblue (B.D.H.), Chlorazol black (I.C.I.), Cottonblue (M & B), Vital new red (B.D.H.), Acid Fuchsin (B.D.H:), Violamin 1A 3 B (Bayer). Acid Fuchsin and Cottonblue in 0,05 % sol. are most suitable for nematodes. Trypanblue in 0,2 % sol. is excellent for bacteria and fungi, but nematodes become too darkly stained. The other 3 dyes give intermediate results. The following dyes failed: Alizarin, Alkaliblue 6 B, Anilin-violet, Azocarmine G, Azur II, Bengal rose, Biebrichs scarlet, Bismarckbrown Y, Boraxcarmine, Brilliant cresyl blue, Brilliant green, Chrysoidine, Congo-red, Dahlia violet, Fast red, Eosin, Erythrosin, Fuchsin, Gentian-violet, Gossypimine, Haematoxylin, Hexanitrodiphenylamine, Indigo-carmine, Janus green, Carmine, Light green, Martius Yellow, Malachite green, Methylene blue, Methylgreen, Methyl-violet BB, Naphtol green B, Naphtol yellow S, Neutral red, Night blue, Nileblue sulphate, Orange G, Phloxin, Rhodanin, Saffranin o, Sudan III, Sudan IV, Thionin, Titan yellow, Tropaeolin 000, Toluidine blue, Victoria blue.

SUMMARY

Four different techniques are described by which it appeared possible to investigate the nematode population in samples of soil and litter quantitatively:

1. a. A staining technique. This was the standard method by which it was possible to isolate practically all nematodes from a given sample.

1. b. Centrifugal flotation: This method took about one eighth of the time required by the foregoing method, but was only slightly less reliable (table 1). This technique is based on the fact that nematodes float in a strong magnesium sulphate solution whereas most soil and humus particles sink. The separation of the floating and the sinking fractions is accelerated by centrifuging. Isolation of the nematodes from the small amount of debris in the counting dish takes little time.

2. a. The isolation of nematodes from oak forest litter had to be done in quite another way. The Baermann technique gives an incomplete separation and little reliability. Highly reliable results were obtained by placing a sample in water on a piece of coppergauze in a beaker, and shaking for 1 minute followed by 4 minutes of rest, during 24 hours. The number of nematodes caught after 24 hours had to be multiplied by the factor 1.25 to obtain approximately the number of nematodes orginally present in the sample.

2. b. The number of nematodes still present in a litter sample after a shaking period of 24 hours was determined after bleaching this material with 6% hydrogenperoxyde to which some ammonia was added until the leaves were yellowish white. Then followed staining with a hot solution (60-70° C) of a dye dissolved in lactophenol. Acid fuchsin or cottonblue were more suitable for staining nematodes while trypanblue was found best for bacteria and fungi.

The author wishes to thank Mr. G. C. Moore and Mr. F. G. W. Jones for assistance with English.

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(*Nematologica*, Vol. I, 1956, no. 3)

THE SEASONAL EMERGENCE OF LARVAE FROM CYSTS OF THE BEET EELWORM, HETERODERA SCHACHTII SCHMIDT *)

BY

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Counts of cyst contents of two different beet eelworm populations in fallow soil during 1954 showed that there was a high rate of larval emergence during April and May with no appreciable emergence from June to September (Fig. 1a). In 1955 an experiment was set up to examine this problem further. Batches of 100 cysts in sand were placed into each of 96 containers made of phosphor-bronze wire mesh. Six containers were then placed into each of 16 large pots, 8 of which contained clay loam and 8 sandy loam. The pots were then buried in soil up to the rim. Sugar beet seed was sown in 4 pots containing clay loam and in 4 containing sandy loam. The seedlings were eventually thinned out to leave one plant per pot. Every month from April to September one container was removed from each pot, the cysts extracted and the egg and larval contents counted. A count of the cyst contents was also made in March before the experiment commenced. By this means it was possible to follow the rate of emergence in 2 soil types, with and without sugar beet from March to October. The difference between the rates of emergence in fallow pots and in pots containing beet plants was negligible in both soil types and so the results have been combined to show larval emergence in the 2 soil types only (Fig. 1b). There was a high rate of emergence during April and May followed by a low emergence rate from June to October, a similar result to that obtained in 1954. The object of this paper is to show that low soil moisture content was probably the chief factor inhibiting larval emergence during the summer of 1955. Further observations on larval emergence in the field combined with recordings of soil moisture are needed to determine whether this is a regular

*) Received for publication: Nov. 2, 1955.

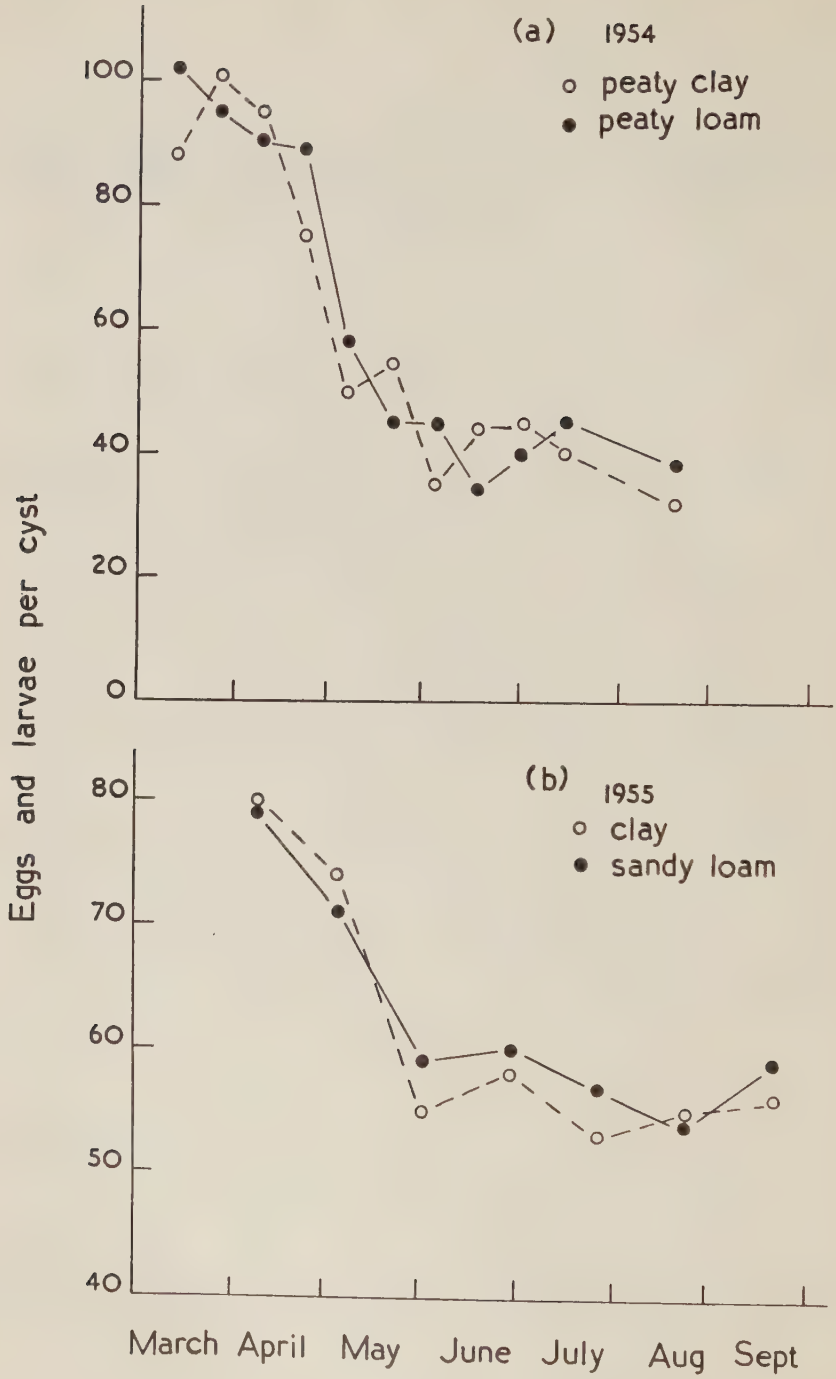

Fig. 1. Variations in the numbers of eggs and larvae in beet eelworm cysts in four soil types during 1954 and 1955.

occurrence. The influence of soil moisture on larval emergence from cysts lying between crumbs or sand particles has been described previously (WALLACE 1954, 1955a, 1955b) but no account was taken of cysts inside crumbs. Different soil types were, therefore, examined to determine the proportion of the cyst population which occurred within soil crumbs and a comparative study was then made of larval emergence from cysts within and between crumbs.

THE DISTRIBUTION OF CYSTS IN THE SOIL IN RELATION TO SOIL CRUMBS

Five samples of soil were taken from each of 4 soil types and allowed to dry in the air for one week. The samples were then separated into 4 crumb sizes with sieves of 10, 20 and 30 meshes per inch. The number of cysts in the different sized crumbs was then obtained by heating the soil in dilute hydrogen peroxide to break down the crumbs and release any contained cysts. Finally the cysts were recovered by the usual flotation technique. The results are given in Table I. JONES (1945) and WINSLOW (1955) have shown that about 98 per cent of a beet eelworm cyst population passes through a 30 mesh sieve of aperture 0.56 mm. Thus the majority of cysts retained on the 10, 20 and 30 mesh sieves must be contained within soil crumbs. As a first approximation these cysts are considered to form the percentage of the population which are within crumbs. This percentage will be an underestimation since a certain number of cysts within crumbs will pass through the 30 mesh sieve. The mean percentage number of cysts lying within crumbs in each soil type is also given in Table I. Aggregate analysis of soils by dry sieving is open to criticism since the drying of moist soil followed by sieving produces artificial crumbs which do not exist in the soil. Thus it might be expected that the figures quoted in Table I for the percentage number of cysts lying within crumbs are probably too high.

An aggregate analysis of the same four soil types was carried out by a wet sieving method. Undried samples were placed on a 30 mesh sieve and water was allowed to drip onto the soil. The numbers of cysts in the soil retained on the sieve and in the soil passing through the sieve were then determined by the method already described. Wet sieving by this means tends to break down the crumb structure and release contained cysts. Thus the estimated numbers of cysts lying within crumbs is probably too low.

Table I

Distribution of cysts in soil—dry sieving—number of cysts/100g soil

	Fine Sandy Loam (1)					Fine Sandy Loam (2)					Peaty clay (1)					Peaty clay (2)				
Replicates	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Sieve size meshes/inch																				
> 10	19	17	12	28	23	21	11	14	9	10	31	47	32	43	47	141	78	54	117	69
10-20	7	11	7	18	12	15	3	13	14	14	25	15	19	17	27	139	161	57	34	74
20-30	17	31	10	17	13	46	24	36	31	37	140	118	140	136	148	124	270	60	36	144
< 30	8	21	27	7	23	7	10	7	5	10	37	62	127	112	86	128	311	272	280	130
Total	51	80	56	70	71	89	48	70	59	71	233	242	318	308	308	532	820	443	467	417
Mean Percentage Number of cysts retained on the 10, 20 & 30 sieves			*73.8*					*88.4*					*69.9*					*58.2*		

Table II

Distribution of cysts in soil—wet sieving—numbers of cysts/100g. soil

	Fine sandy loam (1)					Fine sandy loam (2)					Peaty clay (1)					Peaty clay (2)				
Replicates	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
No. of cysts retained on 30 meshes/inch sieve	131	129	112	161	107	113	53	69	112	41	320	116	246	187	148	287	181	213	144	120
No. of cysts passing through 30 meshes/inch sieve	90	113	84	137	74	122	61	72	93	56	387	136	278	203	166	148	63	121	130	151
Total	221	242	196	298	181	235	114	141	205	97	707	252	524	390	314	435	244	334	274	271
Mean Percent No. of cysts retained on 30 meshes/inch sieve			*56.2*					*49.0*					*46.5*					*60.7*		

Table III

Recovery of cysts from 4 soil types—numbers of cysts/100g. soil

Replicates	Fine sandy loam (1)		Fine sandy loam (2)		Peaty clay (1)		Peaty clay (2)	
	Standard Method	Hydrogen Peroxide method	Standard Method	Hydrogen Peroxide method	Standard Method	Hydrogen Peroxide method	Standard Method	Hydrogen Peroxide method
1	37.6	42.4	29.0	53.8	269.5	348.0	240.8	305.3
2	34.4	46.2	30.0	45.0	233.3	290.7	261.4	411.0
3	32.1	52.3	28.8	50.0	179.1	284.9	256.8	322.4
4	43.3	41.3	36.0	63.8	280.6	244.6	202.4	273.3
5	33.5	51.6	40.8	55.1	245.0	263.6	246.4	360.0
Mean	*36.2*	*46.8*	*32.9*	*53.5*	*241.5*	*286.4*	*241.6*	*334.4*
	difference significant		difference significant		difference not significant		difference significant.	

It is concluded that the percentage number of the cyst population recovered by flotation, lying within soil crumbs is somewhere between the two values obtained by wet and dry sieving in each soil, and is probably at least 50 per cent in all 4 soil types (Table II).

Since a considerable proportion of the cyst population in the soil lies within soil crumbs it was thought possible that a certain number of these might not be recovered from soils when cyst counts were made by the usual methods of flotation. Ten 100 g samples of air dried soil were, therefore, taken from each of the 4 soil types mentioned previously. In 5 of the samples the cyst content was counted by the usual flotation method, standardised as follows. The 100 g soil sample was placed in a 1000 ml conical flask with 400 ml of water, shaken 50 times, filled to the top and left for 10 minutes. The cysts were then decanted off and counted. In the remaining 5 samples the soil was first heated with dilute hydrogen peroxide before the cysts were floated and counted. Results from this experiment are given in Table III. A statistical comparison of the means of the cyst numbers recovered by these two methods for each soil type, indicated that a significantly higher recovery was obtained using the hydrogen peroxide method in all the soil types except peaty clay (2). It is concluded that a proportion of floatable cysts are retained within soil crumbs despite vigorous shaking in water; furthermore this proportion may vary with soil type. It is, therefore, suggested that where estimations of cyst population numbers are made, a dispersing agent should be used in the water to break up the crumbs. Heating with hydrogen peroxide is not the most convenient method to achieve this end. "Calgon" is probably more practicable since no heating is necessary.

LARVAL EMERGENCE WITHIN AND BETWEEN SOIL CRUMBS

The rate of larval emergence from cysts between soil crumbs reaches a maximum at the pressure deficiency at which air first appears in the pore spaces (WALLACE 1955b). Under such conditions cysts probably have optimal conditions of moisture and aeration. Cysts within soil crumbs, however, would have a different environment. The small pores within the crumbs would still contain water and aeration would consequently be reduced.

Clay soil was used throughout the experiments. Clay clods were dried, broken down and sieved to give crumbs of about 1-2 mm diameter. The rate of larval emergence from cysts mixed with the crumbs

was then determined using sintered glass funnels as described previously (Wallace 1954). In order to obtain data on the rate of larval emergence from cysts within crumbs, it was necessary to make artificial crumbs of about 1-2 mm diameter containing cysts. A batch of 200 cysts mixed in powdered clay was placed in a sintered-glass funnel and the whole was allowed to wet. Suction was then applied to remove as much water as possible and the resulting mass of damp clay and cysts was allowed to dry for 12 hours. At the end of this time the clay was cut into crumbs of the desired size. When the crumbs were sieved it was found that only about 2-5 per cent of the cysts were still free. The crumbs were then placed in a sintered-glass funnel and the rate of larval emergence determined. Before counting the emerged larvae the crumbs were lightly crushed when wet to release any larvae contained in the crumbs. Difficulty was experienced in counting the larvae since the clay suspension tended to conceal the larvae in the counting dish. However, by taking numerous small aliquots and diluting with water it was found that counts could be made quite easily. The results are shown in Table IV.

Table IV

Larval emergence from cysts of beet eelworm within and between clay crumbs (1-2 mm)

(a) Numbers of larvae emerging from 200 cysts in 7 days at a pressure deficiency of 10 cm. of water.

Replicates	1	2	3	4	5	6	7	8	Mean
Between crumbs	283	345	380	136	313	220	111	148	*242.0*
Within crumbs	3	0	13	16	0	2	1	11	*5.8*

(b) Numbers of larvae emerging from 200 cysts in 7 days over a range of ten pressure deficiencies.

Pressure deficiency (cm. of water)	2	4	6	8	10	12	14	16	18	20
Between crumbs	3	6	7	33	280	28	50	110	15	11
Within crumbs	1	3	0	1	7	3	3	0	0	0

In the first experiment a comparison was made between larval emergence from cysts within and between crumbs at a pressure deficiency of 10 cm of water, with 8-fold replication. This pressure deficiency is the optimum for larval emergence from cysts between crumbs of this particular size. It is clear that larval emergence within crumbs is relatively low. A similar result was obtained in a second experiment using a range of ten pressure deficiencies.

A study was also made of the invasion of cress roots by larvae from cysts within and between crumbs. The cress (*Lepidium sativum* L.) was grown in sand in sintered-glass funnels. In the first part of the experiment cysts and clay crumbs were mixed with the sand and in the second part crumbs containing cysts were mixed with the sand. The size of the sand particles was 1-2 mm and a pressure deficiency of 10 cm of water was applied. The results (Table V) tend to support the hypothesis that the rate of larval emergence from cysts is relatively low within crumbs, but it is only indirect evidence, since the clay crumb may act as a barrier to larval migration from cyst to cress root.

TABLE V

Invasion of roots of cress by larvae from 300 cysts of the beet eelworm within and between clay crumbs. Clay mixed in sand of particle size 1-2 mm. Optimum pressure deficiency for emergence 10 cm. of water. Duration of experiment 14 days

	Number of larvae / g. root.								
Replicates	1	2	3	4	5	6	7	8	Mean
Between crumbs	205	306	283	219	157	164	129	133	*199.5*
Within crumbs	81	33	81	116	83	58	82	58	*74.0*

SOIL MOISTURE CONTENT IN THE FIELD

From May to October 1955 daily recordings of soil pressure deficiencies were made using soil tensiometers of the porous pot type. Recordings were made at a depth of 4 inches in 4 soil types sown with sugar beet (Fig. 2). The physical properties of the 4 soil types have been described previously (WALLACE 1956). The instruments did not measure pressure deficiencies above 800 cm of water, and this level is indicated on the graphs by the upper dotted line. Fluctuations in pressure deficiency are similar in all four soils although the fine sandy loam (1) shows greater extremes of pressure deficiency than the other three soil types. Recordings of rainfall and soil temperature at a depth of 4 inches were also obtained to show general trends of climate.

DISCUSSION AND CONCLUSIONS

Although the number of cysts situated within soil crumbs forms a substantial percentage of the total cyst population, experiments

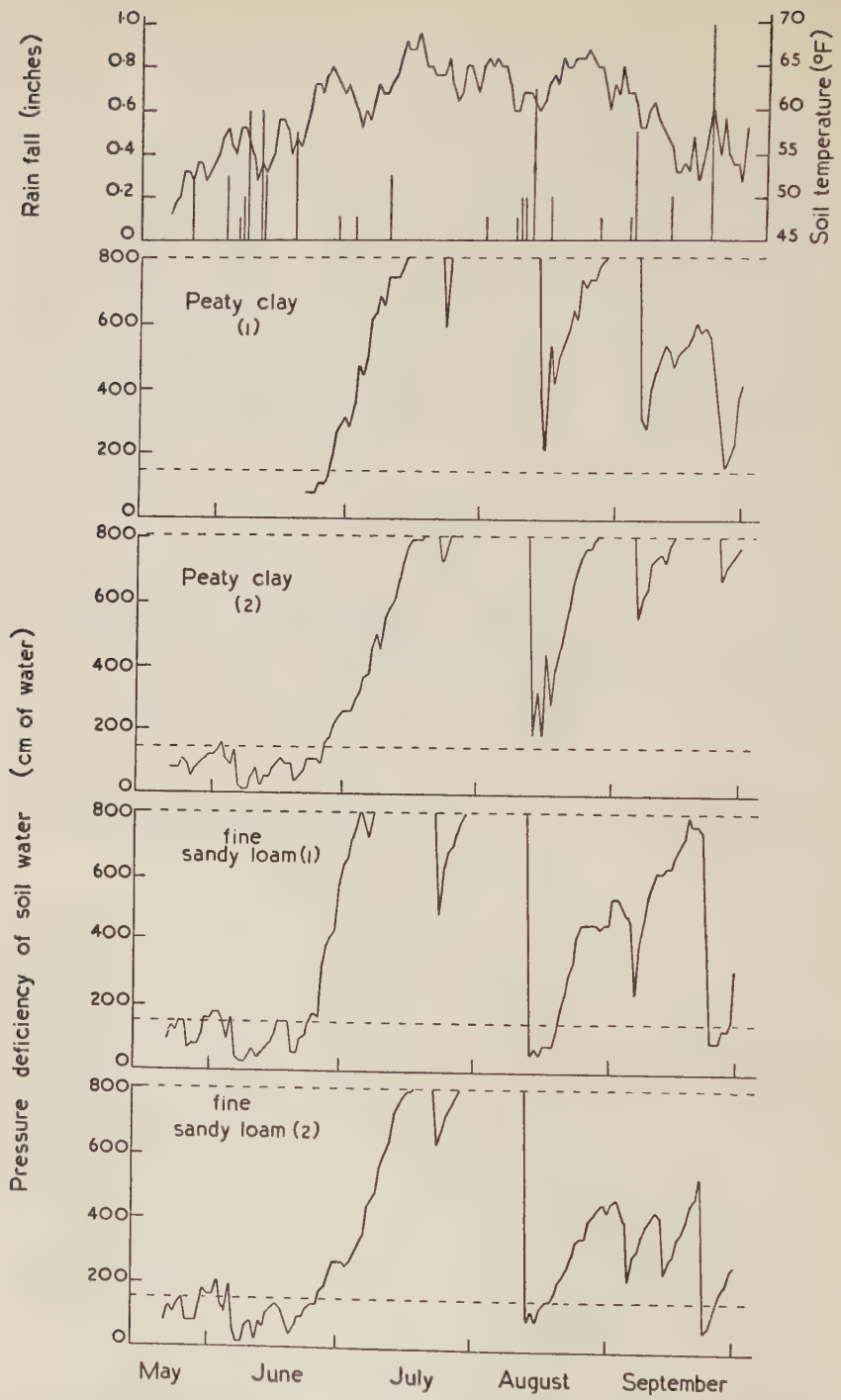

Fig. 2. Fluctuations in the pressure deficiency of four soil types during 1955. The upper graph shows recordings of rainfall (vertical lines) and soil temperature at a depth of 4" (continuous line). The upper dotted line in the other four graphs is the highest pressure deficiency which the tensiometers could record (800 cm of water). The lower dotted line is drawn at a pressure deficiency of 150 cm of water, the point at which it is considered water will have been withdrawn from cysts in the soil, resulting in inhibition of larval emergence.

suggest that the numbers of larvae emerging from them are relatively low when compared with the numbers emerging from cysts between crumbs. It is unlikely that larvae are able to emerge from cysts when the soil is so dry that no free water is present within the cysts, since the larvae must move in a water film to escape via the oral and vulval openings. The pressure deficiency of the soil water at which free water is removed from cysts between crumbs can be calculated theoretically if the dimensions of the openings in the cysts are known. If P is the pressure deficiency in cm of water at which cysts empty, T the surface tension of water (73 dynes per cm) and r the radius of the cyst aperture in cm then $P = \frac{2T}{rg}$ cm of water. The value of r is a variable quantity since in newly formed cysts the oral opening tends to be blocked by mucilage and the vulval opening is a narrow slit. In older cysts the oral aperture may be open and a hole appears on either side of the vulval slit. The diameter of a larva is about 20 μ, so if larval emergence is to occur the diameter of the opening must be at least 20 μ. The pressure deficiency P at which free water is removed from a cyst, the diameter of whose largest aperture is 20 μ will, therefore, be $\frac{2 \times 73}{10 \times 10^{-4} \times 981} = 150$ cm of water approx. In cysts with a larger aperture than 20 μ free water would be removed at a lower pressure deficiency with inhibition of larval emergence. In cysts whose largest aperture was less than 20 μ, free water would not be removed at a pressure deficiency of 150 cm of water, but the larvae would not be able to escape through the narrow opening. Previous experiments on the moisture relations of cysts (WALLACE 1955b) suggested that water was removed from within cysts at about 100 to 135 cm of water pressure deficiency, and that larval emergence approached zero over this pressure deficiency range. It is, therefore, concluded that in soils where the pressure deficiency exceeds 150 cm of water, no larval emergence occurs. In Fig. 2 it will be seen that from the end of June to the end of September, pressure deficiencies in the 4 soil types were mostly above this level. This would account for the low rate of larval emergence during the summer months observed in the field experiments mentioned previously (Fig. 1). Cysts within crumbs may still contain free water at higher pressure deficiencies than 150 cm of water, since water cannot leave the cyst until the water between the particles constituting the crumb has been removed. Thus emergence may still take place

within crumbs but previous experiments (Tables IV and V) have indicated that this is probably negligible.

Jones (1950) suggests that dry soil conditions during July and August may reduce the number of generations and give rise to a periodicity of eelworm attack in beet fields. Observations presented in this paper support this view that soil moisture is an important factor influencing the growth of beet eelworm populations. Larval emergence may continue throughout the summer at greater depths than 4 inches, where soil moisture would be higher, and so it seems desirable that further studies should be made of the effect of pressure deficiency and temperature on larval emergence at various depths over the year.

SUMMARY

Field experiments in 1954 and 1955 showed that there was a high rate of larval emergence from cysts of the beet eelworm during April and May followed by a low emergence rate from June to September. It is shown that low soil moisture content was probably the chief factor inhibiting emergence during the summer months. Larval emergence probably does not occur in soil where the pressure deficiency exceeds 150 cm of water. Recordings of pressure deficiency in 4 soil types during 1955 showed that from June to September the pressure deficiency was mostly above this level. A comparison of larval emergence from cysts within and between soil crumbs was made. At least 50 per cent of the cyst population occurred within crumbs, but the rate of larval emergence from these cysts was very low. It is suggested that when counts of cyst contents in soil are made, a dispersing agent such as "Calgon" should be used to release cysts enclosed in soil crumbs.

The writer wishes to thank the Sugar Beet Research and Education Committee for providing facilities to carry out this research.

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OBSERVATIONS ON SPECIES OF THE GENUS IOTONCHIUM COBB, 1920 *)

BY

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The genus *Iotonchium* Cobb, 1920 has recently been redefined by T. GOODEY (1953). He redescribed *I. fungorum* (Bütschli, 1873) T. Goodey, 1953 and described *I. bifurcatum* T. Goodey, 1953 for the first time. Together with *I. imperfectum* (Bütschli, 1876) Cobb, 1920, the type, these three species then formed the whole of the genus. This genus is characterised by large L-shaped spicules and a tri-lobed head in the male and, in the female, a rudimentary ovary and a uterus which acts as a receptacle for spermatozoa after copulation. The females probably become parasitic in insects. Those figured by BÜTSCHLI (1873 & 1876) were excluded by T. GOODEY from the genus *Iotonchium* and should probably be referred to the genus *Neotylenchus* of Steiner, 1931. All three species were found in association with toadstools.

In 1954 MEYL published descriptions of three more of these fungal associates. (GOODEY's paper had not been published when MEYL's went to press.) They had the same type of spicules but the details of the male head structure and of the female gonads were very different from those described by T. GOODEY. MEYL reported that the ovaries began just posterior to the nerve ring. He called his species *Hexatylus macrospiculatus, Iotonchium cephalostrictum* and *I. mycophilum.*

The inconsistencies between the descriptions of T. GOODEY and of MEYL were so striking that it seemed desirably to investigate the matter and, if possible, to reconcile the differences. Dr. MEYL still had a few specimens available which he very kindly sent to the writer. There were five females and one male of *Hexatylus macrospiculatus,* three females and four males of *Iotonchium cephalostrictum* and two females of *I. mycophilum.* These specimens were processed by the cotton-blue lacto-phenol method (FRANKLIN & J. B. GOODEY, 1949) and mounted in lacto-phenol. Drawings were made and sent, together

*) Received for publication: January 15, 1956.

with the preparations, to Dr. MEYL who agreed in general with the writer's interpretations. Dr. MEYL suggested that the writer should publish his comments on the matter. Later the specimens were remounted by processing into glycerine by BAKER's method (1953) and by ill-luck the only adult male of *I. cephalostrictum* was lost.

A re-reading of BÜTSCHLI's original papers revealed the following situation. The legend to fig. 7c of plate 24 in his 1876 paper on [*Iotonchium*] *imperfectum*, reads: "Kopfende des Weibchens in seitliche Ansicht", but in the text on p. 363 he says: "Kopfende des Männchens mit einigen ziemlich deutlich hervortretenden, papillenartigen Bildungen", and in his 1873 paper, after saying that the lips of the female are not clearly defined, he goes on: "... dagegen beobachtete ich bei den Männchen stets deutliche Spuren von Lippen; einmal schienen mir deutlich drei sichtbar zu sein". It appears therefore, that the legend to fig. 7c is erroneous and should probably read: "Head end of male in ventral view". It certainly appears to be such by comparison with figs. 1b & 2 b of T. GOODEY (1953). It is curious that T. GOODEY should have overlooked this point for from his text he clearly assumed that BÜTSCHLI's fig. 7c represented the head end of the male.

Only three of MEYL's fifteen specimens were fully adult. These were one male (Fig. 2 A-C) and one female (Fig. 2 D-F, I) of *Hexatylus macrospiculatus* and one male of *Iotonchium cephalostrictum* (Fig. 1 D-F). Examination of the males indicated that MEYL had incorrectly interpreted the shape of the head, for these specimens had, in fact, the typical tri-lobed form of head (Fig. 1 E and F). Examination of the pre-adult larval males of *I. cephalostrictum* showed that they were precocious in that each had a fully developed testis and spicules but no bursa; the head, too, was radially symmetrical (Fig. 1 A-C). It seemed, therefore, that this difference, provided that the adult and immature males were one and the same species and there seemed no reason to doubt this, could only be explained by assuming that at the last moult a bursa was formed — which is quite normal of course — but also that the head shape changed from a radially symmetrical to a bi-laterally symmetrical form, flattened in the dorso-ventral plane. It has been possible to confirm this hypothesis by examining the residue of T. GOODEY's preserved but unmounted specimens of *I. fungorum*. Among these specimens were male larvae. Each of them had spicules and a fully developed gonad, no bursa and radially symmetrical head (Fig. 1 L). One specimen, however, was

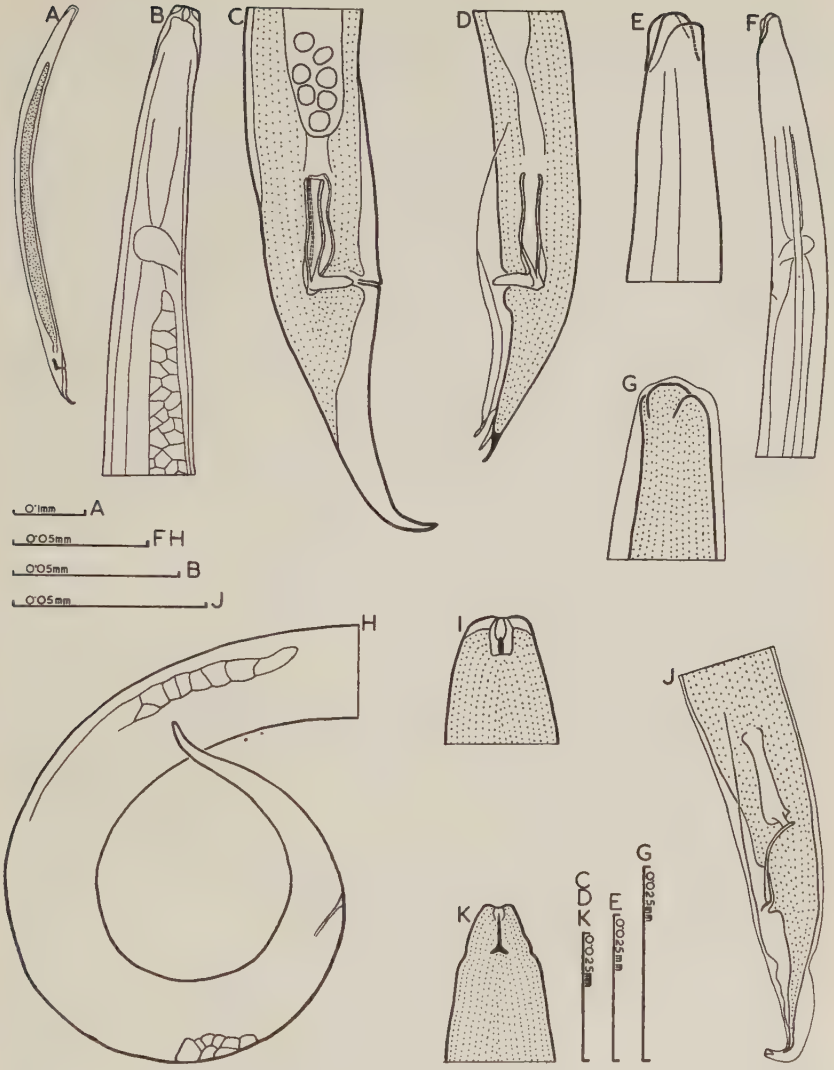

Fig. 1. A-F, H, K. *Iotonchium cephalostrictum*. A-C: Pre-adult ♂. A: whole eelworm showing radially-symmetrical head, mature gonad and spicules but no bursa. (Shrinkage from body-wall at head and tail). B: head of same to show relationship of gonad and nerve ring. C: tail of same. D-F: Adult ♂. D: tail with bursa. E: tri-lobed head. F: fore part of eelworm to show relationships of lateral field, deirid, nerve ring, hemizonid and excretory duct. H: Pre-adult ♀, tail end showing positions of anterior end of ovary, vulva (group of cells) and anus. K: head of pre-adult ♀. G, I, J. *Iotonchium fungorum*. G: head of moulting pre-adult ♂ in latero-ventral view, showing tri-lobed adult head within cuticle of radially-symmetrical larval head. I: head of pre-adult ♂, radially symmetrical. J: tail of moulting pre-adult ♂, the adult form with bursa still within larval cuticle.

moulting, the adult being still within the larval cuticle. It had a bursa and the new head was of the tri-lobed form (Fig. 1 G and J).

As defined by T. GOODEY, the female, after copulation, is found to have a large uterus full of sperms and a rudimentary ovary consisting of a few cells. MEYL considered that the ovary began not far behind the nerve ring in the region of the fore end of the intestine and his figs. 12d and 13a indicate this. In the specimens examined there appeared to be strongly defined cells in that region but they were not part of the gonad. There were ten females among MEYL's specimens only one being adult. This one had its uterus filled with sperms. The ovary in this and in all the other nine immature females, which appeared to be fourth stage larvae since the vulva was not open and, indeed, was not yet completely formed, consisted of a row of five to ten cells located between 50% and 66% of the body length.

Another feature which calls for comment is the spear. T. GOODEY depicted that of the male as a minute point in the mid-ventral line. BÜTSCHLI figured a similar structure. The spear was not discernible in MEYL's specimens. T. GOODEY showed the female spear to be Tylenchoid with the barest suggestion of thickenings at the base. In *I. fungorum* the spear is 15 μ long and 20 μ in *I. bifurcatum*. In the only adult female among MEYL's specimens the spear is 16.5 μ long and is similar in shape to those just mentioned (Fig. 2 D). It is interesting that the spear of the pre-adult larva is c. 10 μ long and, as far as can be made out, appears to be similar to that of a *Hexatylus* (see fig. 1 K). In fig. 11a of MEYL the spear of *H. macrospiculatus* is of this shape. The writer is of the opinion that this is not the spear of the adult but of the pre-adult larva.

With these comments and observations it is now possible to amend the description of MEYL's three species and to supplement the definition of the genus *Iotonchium*. The paucity of the material available does not make it possible to redescribe the three species adequately; this must wait till further material is found for study.

Iotonchium macrospiculatum (Meyl, 1954) new comb. syn. *Hexatylus macrospiculatus* Meyl, 1954

(Fig. 2 A-F, I).

Male (From MEYL) l = 1.0164 mm. a = 24.42: b = 6.73: c = 12.32.
(1 specimen measured) l = 1.17 mm. a = 23.4: b = ?: c = 21.
Body tapering anteriorly, slightly curved towards the ventral side

when dead. Cuticle finely striated except on the lateral fields which extend from behind the head to the bursa. Lateral fields about one-sixth of body-width wide with ten fine incisures which splay out on the bursa. Deirids present in the centre of each lateral field at the same level as the hemizonid. Excretory pore slightly posterior to this level which is the level of the nerve ring.

The head is flattened dorso-ventrally and is tri-lobed. The mouth spear has not been seen but by analogy a small one is probably present. The oesophagus is slightly constricted where the nerve ring encircles it then it widens again and joins imperceptibly with the intestine. No ducts from glands have been seen joining the lumen of the oesophagus. The unreflexed testis is single and stretches forward almost to the nerve ring. The spicules are more or less L-shaped and, according to MEYL, joined. The proximal portions are about 40 μ long the distal 20 μ. There is a pair of post-anal ventral papillae and the bursa, which is quite large, begins near the head of the spicules and surrounds the tail tip. The phasmids have not been seen.

The pre-adult larva is precocious with the spicules and gonad fully developed. However, there is no bursa nor is the head tri-lobed but is radially symmetrical.

Female (From MEYL) l = 1.4850 mm. a = 30.0: b = 8.18: c = 11.25: V = 84.8%

(1 specimen measured) l = 1.69 mm. a = 32.9: b = ?: c = 11.8: V = 87%.

Anteriorly the body tapers slightly to a rounded head: posteriorly rather more to a sharply pointed tail. The cuticle is finely striated. The lateral fields were not seen as the only adult specimen was in ventral aspect and curved laterally so that it would only lie in this plane when mounted. Excretory pore a short way behind the nerve ring. Hemizonid immediately anterior to the excretory pore and extending between deirids. Mouth spear 16.5 μ long, without knobs or swellings at the base. No ducts from oesophageal glands could be seen joining the lumen of the oesophagus. The oesophagus appears to be slightly constricted at the nerve ring, then it widens out and becomes the intestine without any noticeable junction. The gonad consists of a vulva, circular in ventral view, opening well back on the body, a short vagina, a large uterus filled with sperms, a short oviduct and then a row of about twenty cells, the ovary, which reaches forward to just short of half the body length. No post-vulval mid-ventral

supplement was seen, as is shown by T. Goodey (1953) in his figs. 1j and 2j.

The pre-adult larva has a similar shape but the vulva is not open. The ovary is visible as seven or eight cells at c. 66% of the body length. The larva has a short fine spear with basal knobs, the whole being c. 10 μ long. This spear is very like that of *Hexatylus* in form though it was impossible with the material available to see whether the knobs were bifid. From the state of the gonad the female eelworm probably has to parasitize some insect before becoming mature and before the reproductive cycle is completed. This explanation would fit Meyl's observations that when he maintained the eelworms on fungi there was no evidence of reproduction.

This species is transferred from the genus *Hexatylus* to the genus *Iotonchium* for the following reasons. In the male, the shape of the spicules, the large bursa, the post-anal knobs and the tri-lobed shape of the head, and in the female the structure of the spear and the general form of the gonad are typical of the latter genus.

The species is closest to *I. fungorum* and *I. bifurcatum* but it differs from them mainly in the form of the spicules. The bursa is also not so large.

Iotonchium cephalostrictum Meyl, 1954

(Fig. 1 A-F, H, K)

Male (from Meyl) l = 0.5775 mm. a = 19.44: b = 7.00: c = 10.29.

(3 pre-adult larvae measured) l = 0.61 mm. a = 22.2: b = ?: c = 12.6.

(Adult male not measured before being lost).

Body slightly arcuate ventrally when dead. Cuticle finely striated except on the lateral fields. Lateral fields occupy about a sixth of the body-width, beginning not far behind the head and extending to the bursa. Each bears ten very fine incisures, which were only just visible by incident illumination and which splayed out on the bursa. The head is dorso-ventrally flattened and tri-lobed. The spear was not seen. A prominent deirid occurs in the centre of each lateral field shortly behind the nerve ring. The hemizonid is at the same level with the excretory pore immediately posterior. The oesophagus narrows at the nerve ring then widens and becomes the intestine. The gonad is single and stretches as far forward as the nerve ring. The spicules are L-shaped (fig. 1 D), the proximal arm being c. 22 μ long and the distal arm c. 10 μ. The bursa is large, beginning anterior to the

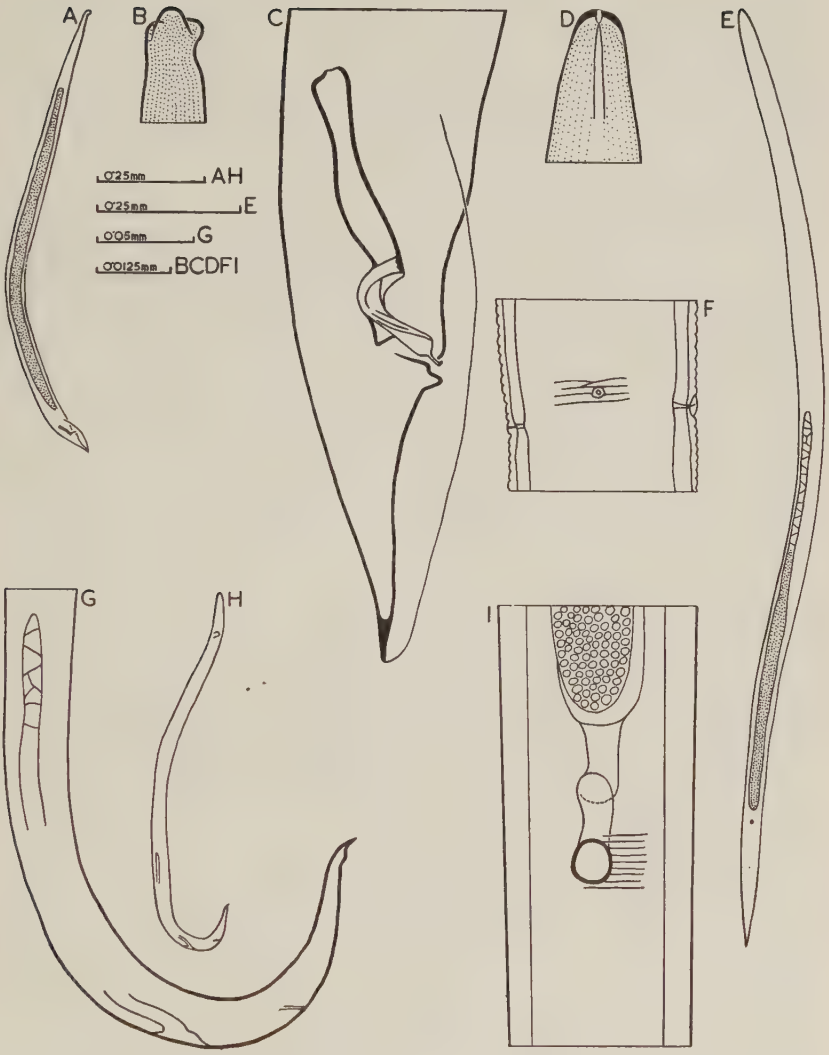

Fig. 2. A-F, I. *Iotonchium macrospiculatus*. A-C: adult ♂. A: whole eelworm. B: tri-lobed head of same in semi-dorsal view. C: tail of same. D-F, I: adult ♀. D: head end to show shape of spear. E: whole eelworm in ventral view. Short ovary, uterus full of sperms, vulva and anus. F: ventral view of excretory pore, deirids and annulation. I: vulval region. Base of uterus full of sperms, looped vagina leading to circular vulva. G, H. *Iotonchium mycophilum*, pre-adult ♀. G: tail end to show extent of ovary, positions of vulva and anus. H: whole eelworm showing same and nerve ring.

spicules and surrounding the tail tip. In the specimen available the distal ends of the bursa were separate from the tail tip but this may

have been an artifact. No post-anal papillae seem to be present and phasmids have not been seen.

The pre-adult larva is precocious, having a fully developed testis and spicules. It has no bursa and the head is radially symmetrical. Female (from MEYL) l = 1.1352 mm. a = 43: b = 7.64: c = 13.23: V = 87.8%.

No adult specimen available.

According to MEYL: — Body thin, averaging twice the length of the male. Tapering anteriorly to a rounded head which is without lips. Tail long and conical with a bluntly rounded tip. Mouth stylet pointed asymmetrically and with dissimilar knobs, up to 23 μ long, with frequent signs of degeneration (?) as malformations (MEYL's fig. 12 f. 1 and 2). Stylet form similar to that of *Styctilus asymmetricus* Thorne, 1941. Oesophagus tubular till near beginning of the intestine, then widening and passing without change into the latter. Behind the stylet bases are fine chitinous rods of about the same length as the stylet.

From observations of the pre-adult larvae that were available it is clear that the gonad is typical of *Iotonchium* and is not as described by MEYL. The ovary has been seen as a row of five or six cells at c. 66 % of the body length. In the pre-adult larva the spear is c. 10 μ long and is of the *Hexatylus* type but the possible bifid nature of the basal knobs could not be determined. The spear of the adult female needs further study to determine whether this species is aberrant in this feature.

This species is an *Iotonchium* on account of the tri-lobed dorso-ventrally flattened head, the L-shaped spicules and the large bursa of the male coupled with the precocious state of the pre-adult larva. The female most probably has the type of gonad characteristic of the genus.

This species is most like *I. imperfectum* but differs in the form and shape of the spicules.

Iotonchium mycophilum Meyl, 1954
(Fig. 2 G-H)

Female (from MEYL) l = 1.9536 mm. a = 53.81: b = 8.45: c = 15.57: V = 87.84 %. No adult specimen available.

According to MEYL: — Body tapers anteriorly to level of the stylet base and is then cylindrical to the bluntly rounded head. No lips or papillae were observed. Posteriorly the body tapers gradually to the

blunt tail tip with the last fifth of the tail cylindrical. The cuticle is thin but clearly annulated. The head is not annulated. Stylet 16-17 μ long with asymmetrical swellings as in female of *I. cephalostrictum.* Oesophagus more or less tubular, swelling out at the level of the nerve ring and passing into the intestine. Three gland nuclei clearly seen in this region. Excretory pore obvious. The granular beginning of the intestine was taken for the determination of the ratio "b". Vulva lips in the form of streamlined brackets {braces} not quite as long as corresponding body width.

Only two pre-adult female larvae were available to study but from them it was clear that the gonad was of the typical *Iotonchium* form, a row of seven or eight cells, the ovary, was seen, in one case at c. 50 % and in the other at c. 66 % of the body length. The structure of the stylet was not discernible.

The form of the vulva appears similar to that described by T. Goodey for both *I. fungorum* and *I. bifurcatum* where there is a flap of cuticle extending posteriorly over the vulva from its front edge.

Males were not found by Meyl.

TAXONOMY

To the definition of the genus *Iotonchium* by T. Goodey may now be added the following points:

1. The pre-adult male larva is precocious, having fully developed spicules and gonad but no bursa.

2. The head of the larval male is radially symmetrical becoming tri-lobed and flattened dorso-ventrally at the final moult.

3. No ducts from oesophageal glands can be seen, in either sex, to join the lumen of the oesophagus.

Tentatively it appears that:

4. The form of the stylet in the female changes at the last moult.

DISCUSSION

The five species of *Iotonchium* in which males have been described appear to be separable into two groups. In *I. imperfectum* and *I. cephalostrictum* the spicules are L-shaped but have no posterior extensions capable of protrusion through the cloaca, nor do there appear to be any post-anal papillae. The other three species *I. fungorum, I. bifurcatum* and *I. macrospiculatus* are alike in having distal extension of the spicules which protrude, or are capable of protruding, through the cloaca. They also have post-anal knobs.

In redefining the genus *Iotonchium* in 1953, T. Goodey set up the following scheme:

Order: Tylenchida Thorne, 1949
Superfamily: Tylenchoidea Chitwood & Chitwood, 1937
Family: Allantonematidae Chitwood & Chitwood, 1937

Subfamily: Iotonchinae T. Goodey, 1953
Genus: *Iotonchium* Cobb, 1920 (emend. T. Goodey, 1953)
Type species: *Iotonchium imperfectum* (Bütschli, 1876) Cobb, 1920
syn. *Tylenchus imperfectus* Bütschli, 1876
Other species: *Iotonchium fungorum* (Bütschli, 1873) T. Goodey, 1953
syn. *Tylenchus fungorum* Bütschli, 1873
Iotonchium bifurcatum T. Goodey, 1953
to which can now be added:
Iotonchium macrospiculatum (Meyl, 1954) n. comb.
syn. *Hexatylus macrospiculatus* Meyl, 1954
Iotonchium cephalostrictum Meyl, 1954
Iotonchium mycophilum Meyl, 1954

T. Goodey set out his reasons for relating the genus to the higher categories. Among those he gave for including the genus in the superfamily Tylenchoidea was the occurrence in both sexes of deirids and a hemizonid. The Tylenchoidea are characterised primarily by the fact that the dorsal oesophageal gland orifice opens in the precorpus. It seems relevant to point out that in not one of the species of *Iotonchium* have any gland openings into the lumen of the oesophagus been seen.

It is obvious that more study is required on the species of this peculiar genus of nematodes, both from the point of view of increasing our detailed knowledge of their morphology and life history and, more particularly, of their probable association with insects. When this knowledge is available it will be possible to determine with a more reasonable degree of certainty the taxonomic position of the genus.

SUMMARY

Iotonchium macrospiculatus (Meyl, 1954) n. comb., *I. cephalostrictum* and *I. mycophilum* are redescribed from Meyl's original specimens. They conform to the characters of *Iotonchium* as set out by T. Goodey (1953). Additional characters of the genus are: (1) that the pre-adult male larva is precocious in having spicules and a fully developed gonad but no bursa; (2) the radially symmetrical head of the larval male becomes tri-lobed and flattened dorso-ventrally at the final moult; (3) no ducts from oesophageal glands can be seen, in either sex, to join the lumen of the oesophagus.

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THE QUANTITATIVE EXTRACTION OF NEMATODES FROM SOIL

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INTRODUCTION

Several methods have been devised for the determination of numbers of eelworms in soil samples: direct microscopy (STÖCKLI, 1944), the Baermann funnel method and variations of it (OVERGAARD NIELSEN, 1949), COBB's decanting and sieving method (COBB, 1918, GOODEY, 1949), a combination of the latter and the Baermann funnel method (CHRISTIE and PERRY, 1951), methods replacing the decanting by a separation by means of an upward current of water (COBB 1924, SPEREITER 1953, OOSTENBRINK 1954) and centrifugal flotation of soil suspensions in a sugar solution (CAVENESS and JENSEN, 1955). The first two methods are for different reasons unsatisfactory. They were discussed by the writer in an earlier paper (SEINHORST, 1950). According to LOWNSBERRY, LOWNSBERRY and MAI (1951) only 30% to 50 % of larvae of *Heterodera rostochiensis* added to soil samples could be recovered by COBB's method. When applied to Dutch soils this method was also found to be inadequate, as a considerable number of eelworms were not recovered from the samples and the fine organic particles in these soils made the separation of the eelworms from the soil particularly difficult. Very good results may be obtained by centrifugal flotation of soil suspensions in a sugar solution (CAVENESS and JENSEN, 1955) but the quantities of soil that can be investigated are generally too small for work on plant parasitic nematodes. Total numbers of eelworms recovered per unit of soil by means of the other methods appear to be generally much lower than those found in similar soils by direct microscopy, centrifugal flotation or the methods described below.

The separation of eelworms from soil by COBB's method is based on the fact that soil particles, with both a lower and slightly higher settling speed in water than eelworms of a certain size, have a smaller

diameter than the meshes of sieves through which the eelworms do not easily pass (diameter of meshes about 0.1-0.05 of length of eelworms), and that soil particles, which have a larger diameter than the meshes of these sieves, have a distinctly higher settling speed in water than eelworms with a length up to 20 times the diameter of these meshes.

A homogeneous suspension of the soil sample to be investigated is made in water. When this suspension is left standing until all soil particles larger than a certain size have reached the bottom of the vessel, a large proportion of the eelworms shorter than 20 times this diameter is still in the suspension together with a proportion of the smaller soil particles. This suspension of the eelworms and small soil particles can be separated from the deposit by decanting carefully. The eelworms are then separated from the small soil particles by sieving the decanted suspension through a sieve with meshes of diameter slightly larger than those of the largest soil particles decanted. The majority of the eelworms remain on the sieve whilst all soil particles pass through. To separate eelworms of various sizes from soil, different settling times and sieves with meshes of appropriate size can be applied successively.

As the eelworms in the suspension do not all have to travel the same distance to the bottom of the vessel, a number settle between the soil particles and remain there whilst the supernatant suspension is being decanted. The separation can be improved by bringing the deposit into suspension again and repeating the process but this tends to become time consuming. Theoretically, therefore, if the settling speed of the nematodes could be reduced to zero or if they could be moved slowly upwards the separation would be much better. With small samples this can be done by using salt or sugar solutions of a high specific gravity.

For large samples a better method is to use an upward current of water. Two types of apparatus based on this principle have been constructed. They both consist of three parts:

1. An erlenmeyer flask (contents 1-2 litre) with a funnel shaped mouth piece;

2. A U tube with a narrow cylindrical leg, inner diameter 5 mm, and a wider partly cylindrical partly conical leg.

3. An apparatus producing a constant flow of water.

The separation of the eelworms from the soil is done in two stages.

First the eelworms and fine soil particles are separated from the coarse particles by elutriation in the apparatus and then the eelworms are separated from the fine soil particles by sieving.

With most soils a sample of 500 grams can be investigated in apparatus of the dimensions shown in the figures.

PREPARATION OF THE SOIL SAMPLE FOR INVESTIGATION

The soil is weighed in a 1 litre beaker, approximately 600 ml of water are added and the soil suspension is stirred until all the lumps are broken down and all the clay is dispersed. With clay soils it may be necessary to soak the sample for a considerable time. Addition of sodium oxalate, which precipitates the Ca, gives a more rapid dispersion. Too much rubbing of the soil is not advisable as this may kill some of the nematodes. After soaking the soil suspension is sieved

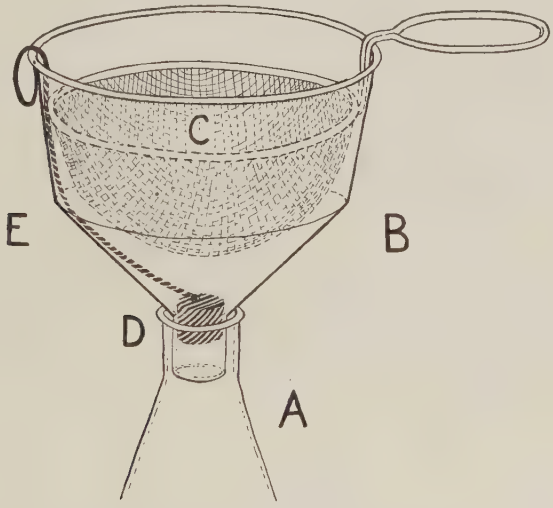

Fig. 1. Sieve and funnel for transferring the soil sample to an erlenmeyer flask. (A) erlenmeyer flask; (B) funnel; (C) 2 mm sieve; (D) stopper; (E) string to pull stopper from stem of funnel.

through a 2 mm sieve in order to remove stones and roots which would obstruct the action of the apparatus. To do this a half spherical domestic sieve with meshes 2 mm wide is placed in a suitable funnel (for shape see fig. 1B). The stem of this funnel is closed with a cork (D) to which a piece of string (E) is attached. The funnel with the sieve is placed on a 1 or 2 litre erlenmeyer flask. The well soaked soil suspension is poured on the sieve and the latter is moved up and

down in the water which has run through. All the soil particles then pass through the sieve. Then the cork is removed from the stem of the funnel by pulling string E, and the soil suspension runs into the erlenmeyer flask. Only a small quantity of water is required for washing the remnants of soil from the sieve and the funnel. The mouth piece is mounted on the flask and the whole filled to the brim with water, the stopper (fig. 2a and fig. 4a) is closed, and the flask is inverted and placed in the position shown in figures 2 and 4.

The stopper is opened. If a stirrer is used it is connected to the transmission axle and the motor is started. In the erlenmeyer flask the coarse particles settle rapidly and the finer particles and the eelworms more slowly. The separation of the eelworms from the coarse soil particles is not complete, but further separation takes place when the sand and other soil particles pass through the mouth piece in tube D or tube C. The soil is replaced by water which causes a strong upward current keeping at least a large part of the small particles and eelworms in the erlenmeyer. However, the smaller the soil particles that pass through the narrow tube of the mouth piece the slower is the upward current and the larger the number of eelworms that sink with the soil particles into tubes D or C_1. The stirrer serves to avoid clogging of the mouth piece and may also separate eelworms from small organic particles, fibres and fungal threads. These have a tendancy to become entangled, thus forming agglomerations which have a settling speed far higher than the eelworms. Stirrer speeds of 100-500 revolutions per minute gave good results. A further separation of eelworms and fine soil particles is effected by an upward current of water in the long conical tube in the apparatus of fig. 2, in the two cylindrical tubes of different diameters in the apparatus of fig. 4. The second type is superior to the first in many respects. However, many data upon which the construction of the second type was based, were collected with apparatus of the first type and it was used in the investigations of several hundreds of soil samples. Therefore it is described at some length here as well.

CURRENT SPEEDS TO BE USED FOR THE SEPARATION OF EELWORMS FROM SOIL PARTICLES.

It has been found that even nematodes as small as 500 μ can be separated quantitatively from suspensions by sieves with meshes of 50 μ wide and 1 mm long nematodes by sieves with meshes of 100 μ.

In all the soils investigated, particles with a diameter of 50 μ to 100 μ had a sedimentation speed of over 350 cm/hour and particles with a diameter of over 100 μ a sedimentation speed of over 700 cm/h. So 350 cm/h and 700 cm/h represented the optimum speeds for the separation of suspensions to be sieved through 50 μ and 100 μ sieves respectively.

By operating the apparatus of fig. 2 at a speed of 250-350 cm/h in the top part of the conical tube 86%-89% (mean of 5 experiments 87,5%) of a number of stem eelworms (length 800-1200 μ) deliberately added to a suspension of 500 grams of soil in water could be recovered and there was no material increase in the number of stem eelworms or *Pratylenchus* recovered when a current speed of 700 cm/h was applied later. Numbers of *Hoplolaimus uniformis* recovered increased considerably, however, when a current speed of 700 cm/h was used after one of 350 cm/h. So for collecting nematodes of this size the speed should be 700 cm/h. Increase of the current speed to 1400 cm/h added no appreciable number of nematodes to those collected at 700 cm/h. The animals collected at this speed were mainly Enchytraeids of lengths up to 1 cm and only a few very large and stout Dorylaims of species, which were already collected in equal or larger numbers at 700 cm/h. The relation of different speeds to the mesh width of the sieves to be used and the size of eelworms collected almost quantitatively has been estimated and recorded in table I. These estimations agree with the results of experiments using different current speeds. Attention is drawn to the fact that the short thick *Criconemoides* must be collected at a speed of 700 cm/hour.

TABLE I

Speed of current	Width of meshes of sieves	Eelworms collected quantitatively	Approximate length
250-350 cm/h	50 μ	Stem eelworms Pratylenchus Aphelenchoides	0.3 — 1.2 mm
600-700 cm/h	100 μ	Hoplolaimus uniformis Mononchus Dorylaims	1.2 — 4 mm
		Criconemoides	0.5 mm
1400 cm/h	250 μ	Large Dorylaims	> 4 mm

Even at a current speed of 250-350 cm/h considerable numbers of large Dorylaims, *Hoplolaimus uniformis* and Mononchs are collected and also at 700 cm/h Enchytraeids 5 mm long.

APPARATUS I

In the conical tube with dimensions shown in fig. 2 the highest speed of the current (at the bottom) is 10-20 times faster than the lowest (at the top). If a soil suspension is now introduced into the top of this tube the small particles and eelworms will be carried off through one of the syphons: larger soil particles and large eelworms stay in the tube at a level, where the current speed is equal to their rate of sedimentation. The coarse sand having a rate of sedimentation even greater than the current speed in the lower end passes through this tube to the vessel E. It would obstruct the action of the apparatus if it stayed in the current.

The water leaves tube D intermittently through one of the two syphons. In this way small soil particles and eelworms are washed from this tube more rapidly than by a continuous flow through a straight lateral tube or over the top edge of tube D. The upper syphon is used as long as the erlenmeyer flask is in the position figured, the lower one when it has been taken from the apparatus. During the first 20 minutes of the separation process a current speed of 250 cm/h is used.

Twenty minutes after erlenmeyer A has been placed on the apparatus all soil particles larger than 50 μ have passed to tube D, but large numbers of the eelworms are still contained inside the flask e.g. about 60% of the stem eelworms present in the soil samples investigated. Therefore, 20 minutes after the beginning of the separation process the erlenmeyer is closed and taken from the apparatus. Its contents are added to the suspension collected from D up to that moment in a container (referred to below as container I). If a stirrer is used, it is stopped 2 minutes prior to the removal of the erlenmeyer as by its action, soil particles larger than 50 μ are kept in the erlenmeyer flask for a considerable time.

After the erlenmeyer has been taken from the apparatus the lower syphon is opened and the apparatus is kept running for another 15-20 minutes at a current speed of 350 cm/h. The suspension running off is collected in container I. If large nematodes must also be collected the apparatus is run for another period of 10 minutes at a current speed of 700 cm/h, the eelworm and soil suspension, which contains

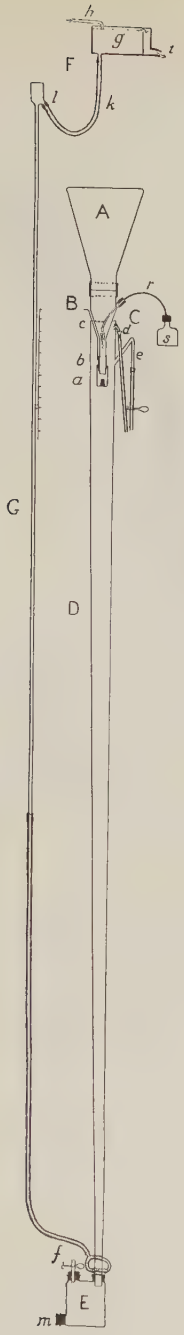

Fig. 2. Apparatus 1 for separating eelworms from soil. Scale 1 : 20. (A) erlenmeyer flask; (B) funnel with (a) rubber stopper; (b) guiding ring and (c) handle for moving guiding ring and stopper; (C) stirring apparatus (see fig. 3 for further explanation); (D) conical tube with (d) and (e) syphons; (E) Woulfe flask with (f) air outlet for easy filling and (m) stopper for emptying apparatus; (F) apparatus to provide constant current with (h): water supply to tank (g), (i): outlet for excess of water, (k): rubber tube and (l) capillary; (G) movable tube to conduct water into (D); (S) dynamo.

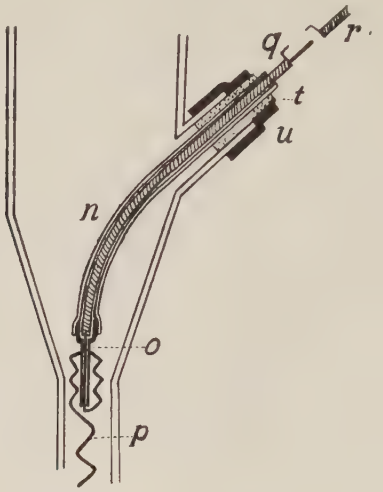

Fig. 3. Construction of stirrer. Scale 1 : 2.
(n) glass, metal or plastic tube; (o) 1 mm hypodermic needle; (p) stirrer bent from 0.5 mm brass or nichrome wire; (q), (r) flexible axle (stainless steel spring) (t) metal tube; (u) rubber collar.

particles between 50 μ and 100 μ diameter being collected in a second container (later referred to as container II).

APPARATUS FOR THE PRODUCTION OF A CONSTANT CURRENT OF WATER

If there were water only in the U tube the speed of the current in the apparatus could be controlled by varying the levels of the water in the narrow and wide limbs of the tube. This simple relationship is disturbed by the varying amount of soil in the apparatus. Thus, by keeping the difference between the levels of the liquid in both tubes constant the current in the U tube would not remain constant. However, an almost constant and easily regulated current speed is obtained with the help of the apparatus F (figs. 2 and 4). In the vessel g a constant water level is maintained by allowing water to enter through h. The excess runs off through i. Water from this small tank is led by the rubber tube k through a hypodermic needle or glas capillary tube l into tube G (figs. 2 and 4). The amount of water flowing into G is now dependent only on the distance between the constant water level in g and the aperture of the capillary l. By varying this distance the output can easily be regulated for all quantities of water that may be required for the apparatus. As long as the output of the apparatus F remains unchanged the current in the U tube will be nearly constant.

It changes very little when the difference between the water levels in both limbs changes.

APPARATUS 2 (fig. 4 and Pl. XXIV, fig. 1)

The main difference between the first and the second apparatus is the different shape of the tube through which the soil passes after leaving the erlenmeyer flask. The preparation of the soil sample is the same and also the apparatus for producing a constant current of water, except that the amount of water used is only about 20 ml/min. and that this amount is constant all through the washing process. This makes a simpler construction possible.

In C_2 a current speed of 350 cm/h is maintained and in D_2 one of 700 cm/h. The diameter of C_2 (2 cm) is about $\sqrt{2}$ times the diameter of D_2 (1,5 cm).

Thus eelworms with settling rates less than 350 cm/h are separated from particles greater than 50 μ in tube C_2 and the eelworms with settling rates between 350 cm/h and 700 cm/h from particles greater than 100 μ in tube D_2. When investigating samples of 500 g, the contents of C_1 and D_1 should be about 200 ml and their width 4-5 cm. Larger soil particles are collected in E.

C_1 and D_1 serve to contain the soil particles, which may pass faster from the erlenmeyer into C_1 than through C_2 and faster from C_2 into D_1 than through D_2. As the inevitable turbulence in tubes C_2 and D_2 results in remixing of soil particles and eelworms of different settling rates these tubes should not be too short. A length of 25 cm for C_2 and of 18 cm for D_2 appear to be sufficient for a good separation. The water leaving the apparatus through tube d is collected in a container (referred to below as container I), as it contains many nematodes.

Twenty minutes from the beginning of the separation process all particles larger than 50 μ have left the erlenmeyer flask and its contents are emptied into container I. It takes another 10 minutes before the separation in tubes C_2 and D_2 is completed. So 30 minutes from the beginning of the separation process tubes C_1 and C_2 are emptied into container I by opening glasscock e and afterwards tubes D_1 and D_2 are emptied into a separate container (container II) by opening glasscock f.

The advantages of the second apparatus over the first are: the shorter washing time, less volume of eelworm suspensions (3-4 l) to be sieved and the shorter apparatus which can be made more easily in glass or plastic.

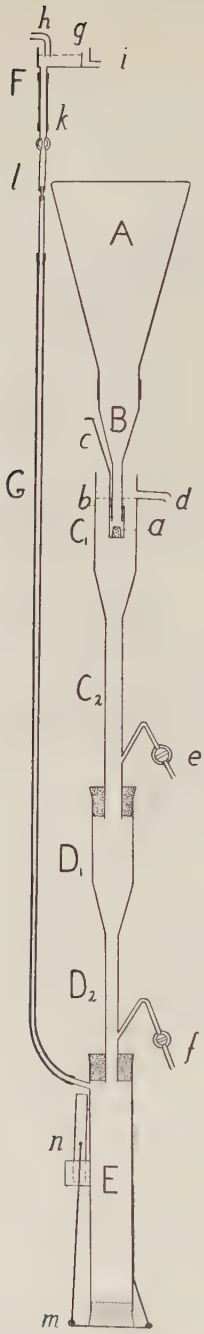

Fig. 4. Apparatus 2 for separating eelworms from soil. Scale 1 : 10. (A) erlenmeyer flask; (B) funnel with (a) stopper, (b) guiding ring and (c) handle to move guiding ring and stopper; (C_1) and (D_1) funnels with (C_2) and (D_2) cylindrical tubes and (d) outlet for water flowing through apparatus during separation process; (e), (f) glass cocks or pinch cocks on rubber tubes; (E) container for collecting coarse particles, (n) handle to move stopper (m); (F) apparatus to provide constant current of water. Rubber tube (k) here provided with glass cock. See fig. 2 for further explanation; (G) tube to conduct water into apparatus.

On a number of occasions the contents of E, after a first separation were put back into an erlenmeyer and allowed to go through the apparatus for a second time. The total number of eelworms collected then was about 10% of the number collected by the first separation and this was also true for both *Pratylenchus* and *Hoplolaimus*. The eelworms collected by the second separation were a representative sample of those collected by the first separation. Thus the loss of about 10% occurring in one separation is, as regards nematodes up to 3 mm long, not due to more rapid individual settling speeds but their tendancy to form entangled masses with fibres and fungal hyphae. These masses settle relatively rapidly. Stirring in the tops of the tubes C_2 and D_2 might improve the separation but the relatively small gain would not make the inclusion of a stirring mechanism worthwhile.

If smaller soil samples are investigated the separation time in the apparatus can be reduced. Thus a sample of 250 g takes about 10 minutes before the erlenmeyer can be emptied and another 5 minutes for completing the separation in tubes C_2 and D_2.

THE SIEVING OF EELWORM SUSPENSIONS.

The contents of container I are sieved through 50 μ sieves, those of container II through 100 μ sieves.

The sievings serve to separate the eelworms from the small soil particles and to diminish the volume of the eelworm suspension.

The ratio of the number of the eelworms which remain on a sieve to the number which pass through, depends on the ratio of the length of the eelworms to the diameter of the meshes, the volume of the suspension, the diameter of the sieve and the time the sieving takes. When 4 litres of a suspension of stem eelworms (length $\pm$ 0,8 mm) were sieved through a 20 cm wide 50 μ sieve, about 75% of these eelworms stayed on the sieve. From the same amount of a suspension of *Pratylenchus penetrans* (length about 0.6 mm) 65% stayed on this sieve, and of *Pratylenchus* larvae of about 0.3 mm 25%. Hardly any adult specimen of *Hoplolaimus uniformis* (1.5 mm) passed through. When the suspension which had passed through the sieve was poured through it a second time about the same percentage of the eelworms of a particular species, which were still in the suspension, were retained on the sieve. The percentage recovery from the suspension by sieving is very nearly expressed by the formula $100\ (1\text{-}a^x)$, where

a = the fraction of the total number of the eelworms of a certain length present in the suspension which passes the sieve and x = the number of times of sieving. The results from series of repeated sievings of suspensions with different eelworms through 15 cm wide 50 μ sieves are presented in table II.

TABLE II

Numbers of nematodes recovered from suspensions by repeated sieving through a 50 μ sieve compared with numbers calculated from the formula 100 (1-a^x)%. Volume of suspensions: 4 litres. Diameter of sieve: 20 cm

Number of times the suspension has been sieved	*Pratylenchus* Average length 250 μ		*Pratylenchus* Average length 500 μ		*Ditylenchus dipsaci* Average length 800 μ	
	Counted	Calculated assuming a = 0.75 T *) = 250	Counted	Calculated assuming a = 0.35 T = 172	Counted	Calculated assuming a = 0.25 T = 154 (Counted before experiment)
1	76	62	122	112	129	115
2	120	109	153	150	144	145
3	147	144	164	165	150	151
4	172	170	169	169		
5	193	190	171	171		
6	209	205				
7	215	216				

*) T = total number of eelworms in the suspension.

The numbers counted, agree fairly well with those calculated and they show only a slight decrease of the effectiveness of repeated sieving. It appears that nematodes can be separated very nearly quantitatively from a suspension by relatively few sievings through a sieve, whose meshes are not wider than 1/10 of the nematode length but that sieves whose meshes are only 1/5 of the nematode length are too coarse for quantitative separation. The percentage recovery after the formula y = 100 (1-a^x) for 3 values of a can be read from the graphs in fig. 5.

In order to investigate the effect of the volume of the suspension and the diameter of the sieve on the recovery of eelworms, suspensions of different volume containing known numbers (274 or more) of *Heterodera rostochiensis* larvae were sieved once through 50 μ sieves

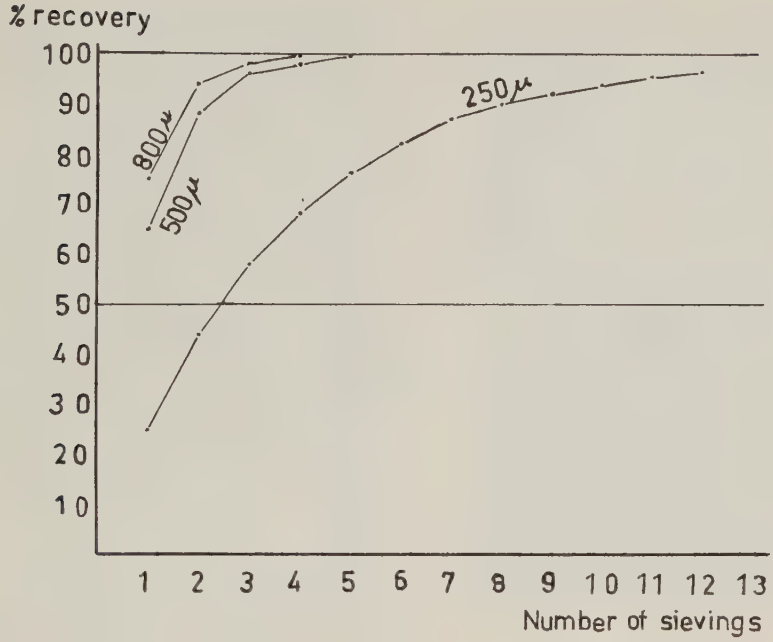

Fig. 5. Graphs showing the relation between the number of sievings through a 50 μ sieve and the percentage recovery of nematodes of three different lengths after the formula: % recovery = 100 ($1-a^x$). Volume of suspension: 4 l; diameter of sieve: 20 cm.

of different diameters. The numbers of larvae retained on the sieves were counted. The results are shown in table III.

TABLE III

Recovery of Heterodera rostochiensis larvae from suspensions of different volume by sieving once through 50 μ sieves of different diameters.

Volume of suspension	Diameter of sieve	% recovered I *)	II *)	mean
200 ml	8 cm	84		84
4000 ml	8 cm	35	46	40
4000 ml	20 cm	66	68	67

*) replicates.

It appears from these results that the volume of suspension to be sieved should be kept as small as possible and that for sieving large volumes larger sieves should be used than for sieving small volumes

because not only does a small sieve become clogged more readily by clay particles than a large one but also a prolonged pouring of water on the same small area causes more eelworms to pass the sieve.

The actual sieving of the suspensions in containers I and II is done as follows. First the contents of container II (250 ml) are sieved repeatedly through a 100 μ sieve, diameter 12-15 cm or through a bank of 5-7 100 μ sieves. The residues on these sieves are rinsed with a gentle flow of water and washed into container I afterwards.

The separation of the eelworms in container I from soil particles and the reduction of the volume of the suspension is done in three stages. The eelworms are separated from soil particles smaller than 50 μ and the volume of the suspension is reduced from 4 litres to about 300 ml in stages one and two. The eelworms are separated from small amounts of fibres and soil particles larger than 50 μ and the volume of the suspension is reduced to about 5 ml in the third stage.

1. The volume of the eelworm suspension is reduced from about 4 l to about 1 l and most of the fine soil particles are discarted by sieving the contents of container 1 repeatedly (5-7 times) through a 50 μ sive, diameter 20 cm or once through a bank of 5-7 50 μ sieves, diameter 20 cm (Pl. XXIV, fig. 2). As the suspension is poured through the sieves the latter are tapped with some hard object to help to prevent the clogging of the meshes by the fine soil particles. The deposits on the sieves are washed into a suitable container. The number of times the sieving of the suspension is repeated or the number of sieves necessary when a bank of sieves is used can be determined with the help of the formula $100\ (1-a^x)$ after having estimated the value of a for the eelworm species to be collected. In order to avoid having too great a volume of suspension when sieving repeatedly through one sieve, or loss of nematodes when a bank of sieves is used, the deposits are only slightly rinsed. Therefore they include some particles smaller than 50 μ in diameter. These are separated from the eelworms in the next stage.

2. The suspension of the deposits of the first sieving (about 1 litre) is sieved repeatedly through a 50 μ sieve, diameter about 12 cm. This time the deposits are rinsed thoroughly with a gentle flow of water to free them from small particles before being collected in a container. It is not advisable to use a bank of sieves in this stage. A prolonged rinsing of the deposit when sieving the first time is especially necessary when it contains small quantities of soil particles larger than 50 μ.

Fig. 1

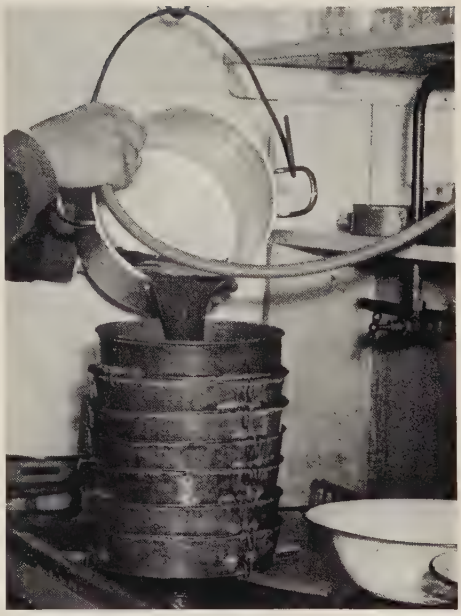

Fig. 2

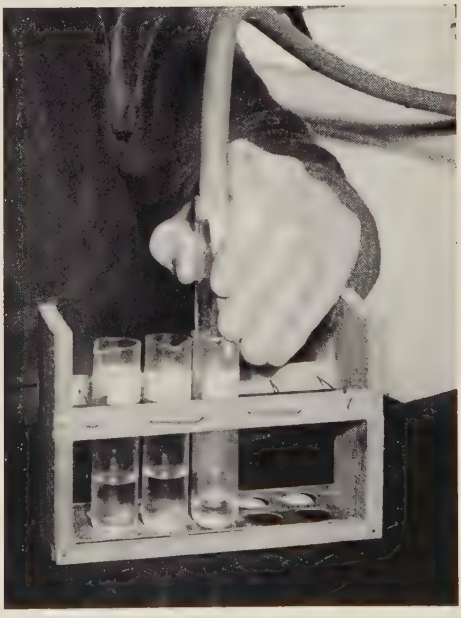

Fig. 3

Fig. 1. Apparatus 2 for the extraction of nematodes from soil, made in glass.
Fig. 2. Sieving an eelworm suspension through a bank of sieves.
Fig. 3. Reduction of the volume of eelworm suspensions in tubes by sucking up excess of water through a filter plate mounted in a plastic tube. Hole in tube is covered by thumb.

When using a bank of sieves this would prolong the rinsing of all sieves and might result in a loss of nematodes. The deposits are collected in about 300 ml of water. They consist of eelworms and a small amount of fibres, organic particles and small mica plates larger than 50 μ. It is hardly possible to avoid the passing of some particles of this size into container I, during the separation process, although with apparatus 2 the amount is nearly always very small. As these particles are a nuisance when the eelworms are counted, a final separation is necessary.

3. The eelworms are separated from small amounts of soil particles larger than 50 μ in diameter by means of a modified Baermann funnel method. The suspension obtained in stage 2 is sieved through a 50 μ sieve diameter 6 cm, having a metal or plastic rim about 5 mm deep and three legs 2-3 mm long. This sieve is then placed in a petri dish and the space between the glass and the sieve is filled with water. The eelworms then pass actively through the sieve.

Some eelworms may pass through the sieve during sieving. These must also be separated from the water passing through the sieve.

An apparatus for rapid sieving of the suspension of eelworms and soil particles and filtering the water running through the sieve is pictured in fig. 6. The sieve B, which is to receive the eelworms and soil particles for the final separation, is placed on supports (a) in a funnel (C). This funnel, having a volume about equal to the amount of suspension to be sieved is provided with a sieve b to support a disc of filter paper.

A cylinder (A) is placed on the sieve B and gently pressed down. The eelworm suspension obtained in stage 2 is now poured rapidly into A: it does not pass through sieve B as rapidly as it is poured into A. So eelworms and soil particles form a well mixed suspension and settle in a even layer on sieve B, which is transferred to a petri dish later. The water, which passes into the funnel, is filtered through the filterpaper disc. The latter retains all eelworms which might have passed through sieve B. The filter paper disc is then placed, face downwards, in sieve B.

Even the most careful rinsing of the deposits on the sieve in stage 2 cannot always free them from all particles smaller than 50 μ. Especially those having diameters only slightly smaller than 50 μ are apt to be kept on the sieves. They tend to pass slowly into the petri dish with the eelworms, however, and are a nuisance during counting. Attemps were made to find a material which, being put on the sieve in

a very thin layer, would prevent these fine soil particles from passing through, but would not be of any hindrance to the eelworms.

Fine glass wool provided a very suitable material after having been processed in water in an homogenizer for about 20 seconds which chopped it into 0.5-2 mm pieces. A teaspoon full of this processed glass wool put into sieve B (fig. 6), before the eelworm suspension is poured in, forms an even layer well mixed with soil particles and eelworms on the sieve when the water has passed through.

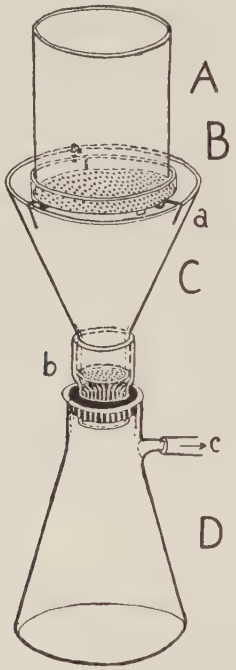

Fig. 6. Apparatus for the final sieving and filtering of the eelworm suspension. (A) cylinder; (B) 50 μ sieve with small legs; (C) funnel; (D) erlenmeyer flask evacuated by air pump; (a) support for sieve; (b) wire or nylon netting to support filter paper disc; (c) tube for connection with air pump.

A thin layer of cotton wool on the sieve proved to have the same effect. Circles of the right size are cut from a sheet of first quality cotton wool. These circles can easily be split into very thin layers. Such a thin circle is placed on the sieve. To avoid tearing when the eelworm suspension is poured, a circle of large mesh plastic or metal netting is laid on the cotton wool.

The quantity of soil particles on the sieves in the petri dishes should

be very small as with thicker layers the eelworms are less active. This inactivity of eelworms in layers of wet soil is the cause of the very unsatisfactory results obtained with the Baermann funnel method and of the more or less incomplete separation obtained when CHRISTIE and PERRY's method is used.

Nearly all the active eelworms pass through the sieve within 4 to 12 hours.

Counting is done in 2 or 4 mm deep counting slides. In order to estimate the degree of infestation of a soil with stem eelworms or *Pratylenchus* all specimens of these species separated from a soil sample of 500 g must be counted as their number is generally lower than 200. In most cases the petri dishes contain more water than the contents of one or two counting slides and so the volume of the eelworm suspension must be reduced to 3-5 ml. This is done by pouring it from the petri dish into a tube (diameter ± 2 cm). As soon as the eelworms have settled most of the water is removed by sucking it up with a pipette, or through a 1,5 cm wide sintered glass filter (e.g. Schott G 3) mounted in a glass or plastic tube, which is connected to a suction pump. The filter plate can be washed free from adhering eelworms with a few drops of water after turning the suction off. If a hole has been drilled in the plastic tube the filter can easily be handled by closing this hole with the thumb or a finger for sucking and opening it when the filter plate is washed (Pl. XXIV, fig. 3). The suspension should not be poured into sintered glass filters as many eelworms are sucked into the pores of the filter plate and cannot be removed easily by washing with a small amount of water.

INVESTIGATION OF SOIL SAMPLES HAVING SMALL QUANTITIES OF TINY ORGANIC PARTICLES.

The methods described above apply to soils with a considerable amount of organic particles of sizes between 50 μ and 200 μ as nearly all Dutch soils.

The investigation of a number of red and grey soils from northern Victoria (Australia) and from near Adelaide by the two erlenmeyer method (SEINHORST 1955) showed that separation at current speeds of 700 cm/h combined with sieving through 50 μ sieves can possibly be applied to them. This would give at least a more rapid and perhaps a more effective separation. Obviously these soils did not contain particles larger than 50 μ with a sedimentation speed below 700 cm/h.

TIME REQUIRED FOR THE INVESTIGATION OF A SOIL SAMPLE.

The time required to prepare a sample for the apparatus (mixing, weighing, making a suspension in water, sieving this suspension and transferring it to the erlenmeyer flask) depends on the nature of the sample. With a sandy soil it takes about 5 minutes, but with clay soils it may take considerably longer as the mixing of the sample is more difficult and the clay disperses slowly.

Setting up the erlenmeyer, starting the separation process and collecting the suspension takes about 1 minute.

The time taken to sieve the eelworm suspensions collected depends on the number of repetetive sievings. Four litres of suspension sieved 7 times takes about 10 minutes, but if a pile of sieve is used the sieving can be completed in 7 minutes.

THE STIRRING MECHANISM

The construction of the stirrer is shown in fig. 3 (p. 256). The transmission axle from the motor to the stirrer is a stainless steel spring to allow for the bends in the axle. There is a connection between the two pieces of this spring q and r near the conical mouth piece. A metal rod is fastened in part q of the spring and protrudes about 1 cm. The end of this spring is bent to a hook. The width of the metal rod is such that spring r can easily be slipped on it. This spring is also provided with a hook which catches the hook on spring q when turning. The straight top of the nichrome wire stirrer p passes through the hypodermic needle o, which serves as a guide, and is fastened to the spring q inside the socket of the needle.

Tube n should be an airtight fit in the mouth piece B but it is not necessary to make the stirrer p an airtight fit into hypodermic needle o. The capillary forces in this part of the mechanism do not allow air to pass through.

Bicycle lamp dynamos of a type which run at not more than 50 r.p.h. on 4 to 6 volts, 50 cycles A.C. proved to be cheap and satisfactory motors. They can easily be attached to a frame and no gears are required. The transmission spring Y is directly connected to the axle of the dynamo.

SUMMARY

Two types of apparatus for the determination of numbers of eelworms in soil samples are described and figured. With both types about 85-90% of the eelworms are recovered from soil samples of 500 g. With the more practicle

of these apparatuses most of the eelworms are collected in about 3 litres of water together with only a very few of or no soilparticles larger than 50 μ. The larger forms are collected in about 0.3 liters of water with hardly any or no soil particles larger than 100 μ. The eelworms can be separated almost completely from these soil particles by sieving the suspensions repeatedly through 50 μ sieves (for suspensions with small eelworms and soil particles not larger than 50 μ) or through 100 μ sieves (suspensions with larger eelworms and soil particles not larger than 100 μ). The sieving technique is fully discussed. Methods for a final separation of the eelworms from small amounts of soil particles larger than 50 μ and for the reduction of the eelworm suspensions are described.

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FURTHER OBSERVATIONS ON THE EFFECTS OF PEAS, BEANS AND VETCH UPON SOIL POPULATION LEVELS OF PEA ROOT EELWORM, HETERODERA GÖTTINGIANA LIEBSCHER *)

BY

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WINSLOW (1955) gave the results of microplot and garden plot experiments in which, in 1954, he tested the effects of various leguminous plants upon the soil population level of pea root eelworm (*Heterodera göttingiana* Liebscher). In his microplot experiment, starting with a uniform initial population of 127 egg/g. air dried soil, peas maintained the initial population, broad bean, lentil and sweet pea caused considerable reductions, while other leguminous plants caused smaller reductions of about one third, comparable with the effects of fallowing. The Aldringham soil was a light sand and, in it, under eelworm attack, the peas grew to a height of 3 in. only and failed. The adjacent garden plots were on heavy soil overlying chalk marl and were inoculated artificially with quantities of Aldringham soil so that the initial eelworm population was low; peas sown on these plots grew normally and gave considerable increase in population. Field bean on these garden plots behaved in the same way as broad bean in the microplots, and caused a decrease.

In 1955, the same plots were utilised, the chief differences being that initial populations were variable, the fertilizer applied contained nitrogen (as well as phosphorus and potash) and the seasonal conditions were reversed in that 1954 was dry at first followed by cool moist weather and 1955 wet and cool at first followed by hot dry weather. In the 1955 microplot experiment, the range of initial populations available (12-136 eggs/g. soil) was divided into six levels. Four varieties of pea (*Pisum sativum*), three varieties of bean (*Vicia faba*)

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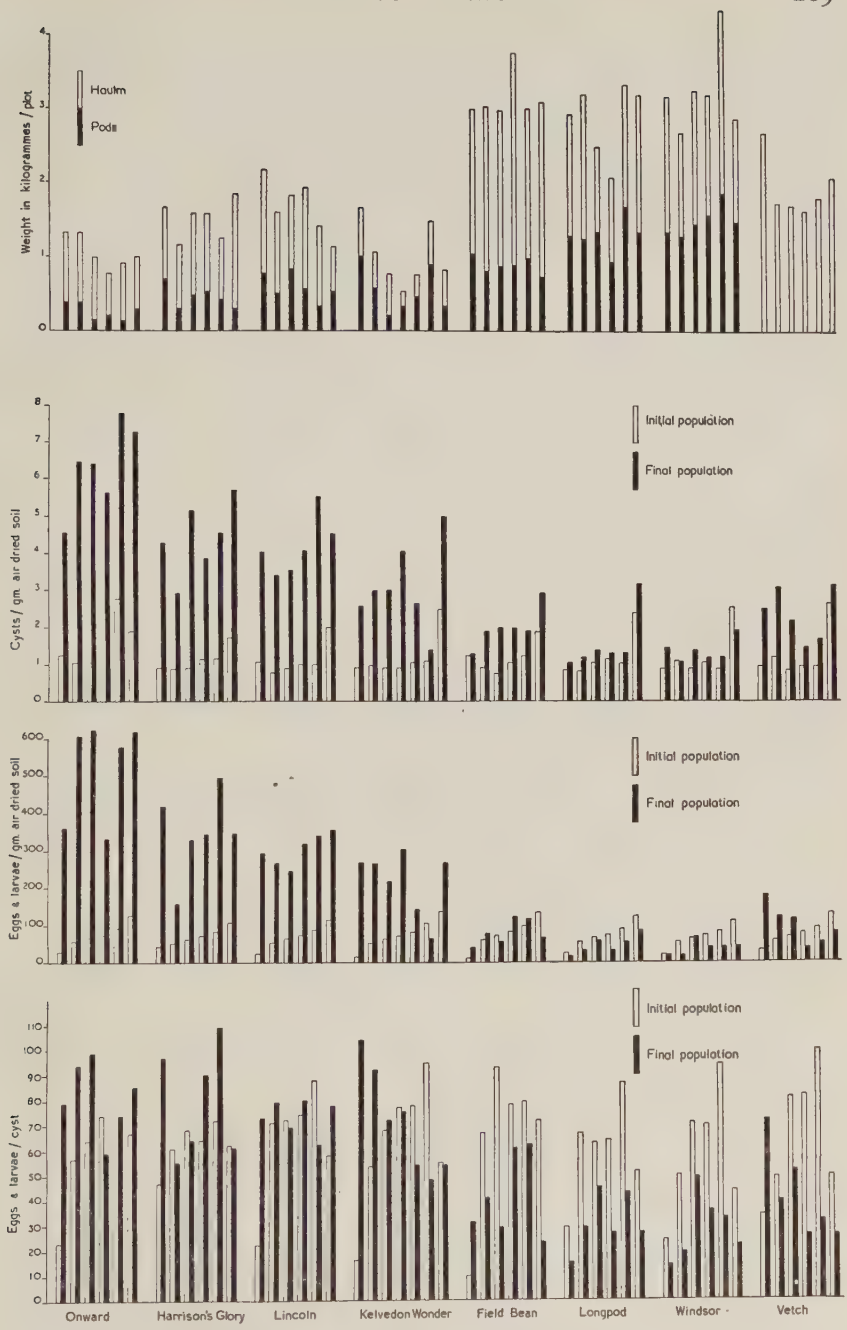

Fig. 1. Results of the 1955 microplot experiment with pea root eelworm. Top row of histograms, plot yields; second to fourth row, eelworm populations in terms of cysts, eggs, and eggs per cyst, respectively.

TABLE I

Comparison of Mean Cyst and Egg Populations in the 1955 Microplot Experiment

Crop	Cysts/100 g.			Eggs/g.			Eggs/cyst		
	Before	After	Increase	Before	After	Increase	Before	After	Increase
Peas									
Onward	150	635	485	75	522	447	53	82	29
Harrison's Glory	113	442	329	71	350	279	62	79	17
Lincoln	112	419	307	70	304	234	64	73	9
Kelvedon Wonder	121	310	189	75	220	145	63	71	8
Average	124	452	328	73	349	276	61	76	15
%	—	—	265 %	—	—	378 %	—	—	25 %
Beans									
Field Bean	118	200	82	77	81	4	67	41	—26
Longpod	122	157	35	73	48	—25	60	31	—29
Windsor	123	137	14	69	39	—30	59	29	—30
Average	121	165	44	73	56	—17	62	34	—28
%	—	—	36 %	—	—	—23 %	—	—	—45 %
Vetch	127	235	108	79	100	21	66	42	—24
%	—	—	85 %	—	—	27 %	—	—	—36 %

and vetch (*Vicia sativa*) were sown in six-fold replication (seven-fold for the pea Kelvedon Wonder), one replicate of each occurring at random in each of the six population levels. All plots were sown in early April and were harvested green in July. At this time, the pea Kelvedon Wonder was practically at the end of its vegetative life but the other peas, the beans and the vetch, were capable of further growth.

The treatment of the garden plots differed from that of the microplots. Each plot was split into two, and one half grew field bean while the other grew pea, variety Onward. Sowing, harvesting and manuring were the same as for the microplots.

RESULTS

The results of the microplot experiment are shown in Fig. 1, and Table I. In Fig. 1, the plots for each crop are arranged in ascending order of initial egg population/g. soil (3rd row open histograms). Peas gave rise to marked increases in the cyst and egg populations of the soil. Vetch doubled the cyst population but gave only a small increase

TABLE II

Comparison of Cyst and Egg Population in the 1955 Garden Plot Experiment

Plot No.	Cysts/100 g.			Eggs/g.		
	Initial Population	After Field Bean	After Onward Pea	Initial Population	After Field Bean	After Onward Pea
1	24	285	331	9	172	237
2	23	109	213	13	69	195
3	39	93	184	47	45	113
4	87	205	253	86	194	192
5	86	168	337	88	71	301
6	88	355	388	91	191	414
7	123	284	399	112	206	430
8	106	53	128	144	13	131
9	172	158	310	155	146	213
10	114	208	299	175	147	188
11	164	209	349	211	57	398
12	199	193	359	218	165	297
Means	102	193	296	112	123	259
Increase	—	91	194	—	11	147
%		89 %	190 %		10 %	131 %
Eggs/cyst				110	64	88
Increase				—	—46	—22
%					—42 %	—20 %

in eggs (27 %). Field bean also nearly doubled the cyst population but barely maintained the egg population (5 % increase). Longpod and Windsor beans caused relatively small increases in cyst populations and reduced the egg populations.

There was no clear evidence of a relationship between initial egg populations and yield, or between yield and final egg populations.

The results of the garden plot experiment are given in Table II. They show greater variability than the results from the microplot experiments because (1) the soil has not been homogenised and (2) it is assumed that the halves of the split plots have identical initial eelworm populations, which is not necessarily so. In the heavier soil of the garden plots, field bean produced somewhat greater final cyst and egg populations than in the light soil of the microplots. Onward pea, however, gave lower final populations than in the microplots, but still relatively much higher than those of field bean. Again there appeared to be no clear relationship between yield and initial or final population.

DISCUSSION

In the experiments described, the effects of peas, beans and vetch upon the pea root eelworm population of the soil are compared under conditions standardised for sowing, harvesting, cultivation and manuring. Under field conditions field bean and vetch might have given somewhat greater increases if allowed to grow over a longer period or if the beans had been sown as a winter crop. Even allowing for this, however, it seems clear that beans and vetch are less efficient hosts of pea root eelworm than peas. This does not mean that the populations supported by beans and vetch would not be injurious to peas in a normal season. Field bean and vetch both produced final populations of the order of 100 eggs/g. of soil while Longpod and Windsor beans produced final populations of about half this value, 48 and 39 eggs/g. respectively, all of which must be regarded as dangerous for peas.

The 1955 results differ from those of 1954 (Winslow 1955) in the higher final eelworm populations produced by peas and field bean. Apart from this there is general agreement regarding the comparative levels produced by different hosts. The relatively lower level produced by vetch in 1955 may, perhaps, be due to the shortened vegetative period.

One striking difference between the 1955 and 1954 experiments was the absence of the typical collapse symptoms in peas in 1955. This difference was probably due to the cool moist conditions prevailing throughout April, May and June, but it may also have been due in part to the inclusion of nitrogen in the fertilizer applied. This may have counteracted the reduced nodule formation usually associated with pea eelworm attack on peas.

The writers wish to thank Dr M. Oostenbrink and Mr B. A. Cooper for confirming the identification of *Heterodera göttingiana.*

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WINSLOW, R. D. (1955). The effects of some leguminous crops on soil population levels of pea root eelworm. Plant Pathology, 4, 86-8.

SUMMARY

In microplot and garden plot experiments, peas caused marked rises in cyst and egg populations of pea root eelworm. Vetch doubled the number of cysts but gave only a small increase in eggs. Field bean also nearly doubled the number of cysts but barely maintained the egg population. Longpod and Windsor beans caused relatively small increases in cyst numbers and reduced the egg populations. Despite the high initial egg populations of some plots, no symptoms of pea 'sickness' appeared in 1955, probably because of the cool, moist weather during the growing period and possibly also because of the nitrogenous fertilizer applied to the plots.

ZUSAMMENFASSUNG

In Mikroparzellen- und Gartenversuchen wurde durch den Anbau von Erbsen ein deutliches Ansteigen der Cysten- und Eipopulationen des Erbsennematoden hervorgerufen. Bei Wicken verdoppelte sich die Zahl der Cysten, aber es gab nur eine geringe Vermehrung an Eiern. Auch bei Ackerbohnen verdoppelte sich fast die Anzahl der Cysten, aber die Eipopulation wurde kaum gehalten.

Die beiden Sorten von Gartenbohnen „Longpod" und „Windsor" verursachten nur ein relativ geringes Ansteigen der Cystenzahlen und eine Verminderung der Eipopulationen. Trotz der anfangs hohen Eipopulationen einiger Parzellen traten 1955 keine Symptome der „Erbsenmüdigkeit" auf, wahrscheinlich wegen des kühlen und feuchten Wetters während der Wachstumszeit und wahrscheinlich auch wegen des den Parzellen verabreichten Stickstoffdüngers.

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DIE BELEUCHTUNG DER PROBESCHALEN BEI DER UNTERSUCHUNG AUF CYSTENBILDENDE NEMATODEN

VON

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Eine gute Beleuchtung der Probeschalen ist bei der Untersuchung auf cystenbildende Nematoden eine unerlässliche Voraussetzung, da besonders über längere Zeiträume hin die Ermüdung der Augen sehr stark ist. In München liegen die Hauptzeiten für die Untersuchung auf Kartoffelnematoden gerade in den Wintermonaten, der Arbeitsraum geht nach Norden; daher wurde nach Möglichkeiten einer günstigen Beleuchtung gesucht (Pl. XXV).

Die Ausleuchtung einer Wasserfläche ohne Schatten und Reflexe bietet natürlich besondere Schwierigkeiten. Zunächst wurden die vorhandenen Leuchtstoffröhren wegen ihres blendungsfreien Lichtes verwendet, doch stellte sich sehr bald heraus, dass das ständig flimmernde Licht auf der leicht bewegten Wasseroberfläche unangenehme Lichterscheinungen hervorruft. — Eine direkte Beleuchtung mit handelsüblichen Lampen war ebenso nicht durchführbar, da hierbei Spiegelungen auftreten, die die Genauigkeit der Untersuchung beeinträchtigen. — Wegen dieser Mängel kamen also nur indirekte Lichtquellen in Frage. Zunächst wurde die Decke des Raumes mit Tiefstrahlern ausgeleuchtet. Dabei zeigte es sich, dass eine über dem Kopf des Untersuchers liegende leuchtende Fläche nicht ausreicht, da nun der Schatten des Untersuchers stört. Je stärker man die Lichtquelle wählt, desto stärker erscheint der Schatten.

Ausgehend von der Überlegung, dass die Fensterflächen senkrecht stehen und damit das Tageslicht waagrecht in den Raum kommt, wurden zwei in den Abb. gezeigte Lampen konstruiert. Hier fällt das Licht einer 150-Watt-Lampe mit ZEISS-IKON-Glasspiegelreflektor auf eine mattierte weisse Kunststoffläche („Trolonit"). Diese Fläche ist grösser als die zur Untersuchung dienenden Suppenteller. Die Teller können nun näher oder ferner von der Fläche stehen, ausserdem ist der Scheinwerfer nach allen Richtungen beweglich. Damit kann jede gewünschte Lichtmenge erreicht werden.

Die beiden beschriebenen Lampen sind seit fast zwei Jahren in Betrieb und haben sich vorzüglich bewährt. Es hat sich sogar herausgestellt, dass durch den gelben Lichtanteil die goldbraunen Cysten von *Heterodera rostochiensis* viel besser zur Geltung kommen als bei Tageslicht, was besonders bei Proben aus schwarzem Moorboden einen grossen Vorteil bedeutet. Die Lampen werden deshalb auch in den Sommermonaten und an hellen Tagen verwendet. Die gewählte Lichtstärke (150 Watt) ist selbst an sehr hellen Tagen ausreichend, um Zwielicht zu verhindern. Eine derartige, bei jeder Untersuchung gleichmässige Beleuchtung verringert die Fehlerquellen naturgemäss ganz bedeutend. Dass individuelle Unterschiede bei verschiedenen Untersuchern auftreten, liegt an der Art der Untersuchung, die ja keine rein objektive, sondern eine mehr subjektive Methode ist. Durch die Beleuchtung, wie wir sie in München nun haben, konnten jedoch diese Unterschiede weitgehend ausgeglichen werden. Ausserdem ist die Ermüdung der Augen sehr viel geringer als bei anderer Beleuchtung.

Vielleicht liesse sich die Konstruktion der Lampen noch etwas eleganter gestalten, doch kommt es ja wohl nur auf ein Prinzip an. Und dieses Prinzip besteht in einer gleichmässig stark leuchtenden Fläche senkrecht vor der Untersuchungsschale. Diese Fläche muss grösser als die Schale, beweglich und mit veränderlicher Lichtquelle versehen sein, um die jeweils optimale Lichtstärke zu erhalten.

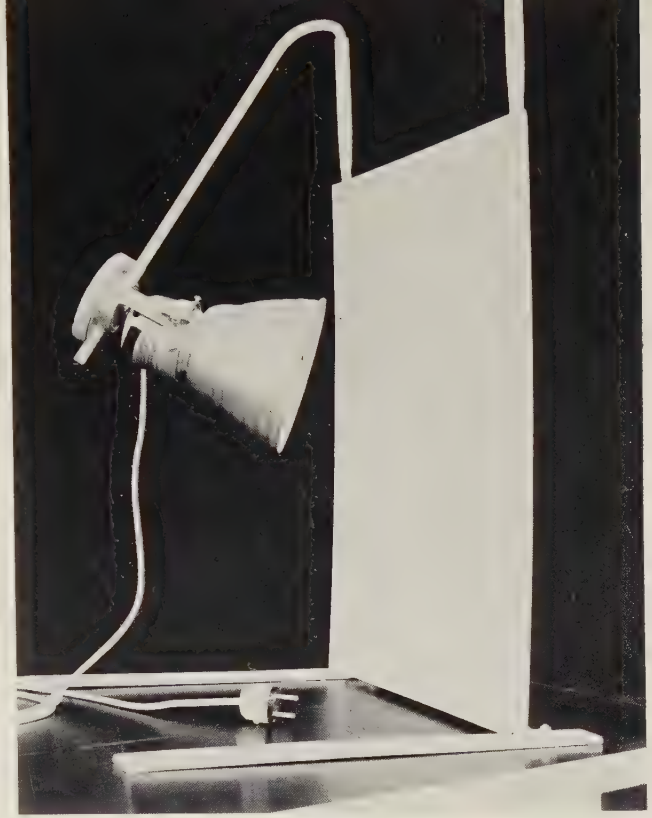

Abb. 1. Lampe zur Beleuchtung der Probeschalen. — Eine weisse, mattierte Kunststoffläche („Trolonit") wird von einer 15-Watt-Lampe mit Glasspiegelreflektor angestrahlt. Der Lampenarm ist nach beiden Seiten, der Reflektor vertikal und horizontal verstelbar. Zwischen die beiden flachen Füsse wird die Untersuchungsschüssel gestellt. (phot. KLEWITZ).

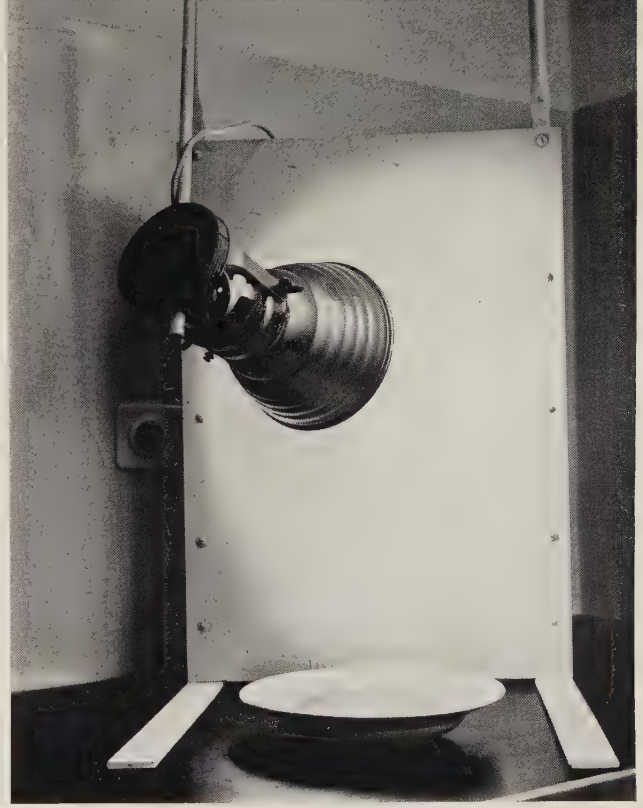

Abb. 2. Die Lampe in Betrieb. — Deutlich zu erkennen ist der Lichtkreis auf der direkt angestrahlten Fläche und die günstigen Lichtverhältnisse auf der indirekt beleuchteten Schüssel. Der Untersucher ist bei der Arbeit nicht behindert, da sich der Scheinwerfer seitlich über seinem Kopf befindet. Die Grössenverhältnisse veranschaulicht die Schale, ein normaler Suppenteller. (phot. KLEWITZ).

NEMATOLOGICAL NEWS

TAXONOMY

Since Nematology is now in a phase of rapid growth, many new taxonomic problems have arisen and it has become evident that there is acute need for a great variety of taxonomic publications summarizing the work of the past, describing new species and developing new concepts for the future. This need can be best met by creation of departments of nematology in established museums. Such departments would also provide facilities for building permanent collections of specimens and literature. It is proposed that this be given serious consideration with the goal of establishing such a department at the earliest opportunity.

V. H. Dropkin, U.S. Dept. of Agric.,
Section of Nematology.

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PERSONALIA

Dr. G. Steiner has retired from the Section of Nematology, US Dept. of Agric., Beltsville (Md.) and will continue his work in nematology at the Experiment Station Rio Piedras, Puerto Rico. His successor is Dr. A. L. Taylor.

A. H. Meyl

(*Nematologica*, Vol. I, 1956, no. 4)

THE CONTROL OF DITYLENCHUS DIPSACI ON OATS *)

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Oat crops are liable to attack by a strain of the stem eelworm, *Ditylenchus dipsaci*, causing a serious disease, "tulip-root", which may reduce the grain yield by 80%. KÜHN and RITZEMA BOS made fundamental studies on the general species; GOODEY (1933) published a full account of the particular disease of oats, and ROBERTSON (1955) has more recently described practical implications of the malady. He showed that the eelworm can remain alive in the soil for a maximum period of four years in the absence of susceptible hosts. There are, however, several hosts, mostly common weeds, which can keep the eelworm population active in the soil for longer periods. Control by adequate rotation is therefore useful but depends for success on freedom from weed hosts. The oat variety Milford (S225) is, according to ROBERTSON, completely resistant, and provides the most practical and economic method of control, particularly on land which has substantial infestation. The variety Milford, however, is not among the highest-yielding varieties, and an economic treatment which could be applied to the higher-yielding crops would still be useful.

In 1948, a farmer in Dumfriesshire kindly placed an isolated plot of ground severely infested with the oat strain of *Ditylenchus dipsaci* at our disposal for investigations on the disease and its control. Cultural operations were done by the farmer after all work was completed on other parts of the farm, when implements could be cleaned before storage. Hygienic separation of the grain for threshing necessitated its transport to Auchincruive, and therefore only small 1,000-th acre plots (6 ft. by 7 ft. 3 in. or 1.83 by 2.21 metres) could be used for estimations of yield. The plots were, however, replicated, usually four times, and gave adequate comparisons of relative yields which also allowed the equivalent performance per complete acre to be calculated. Farm performances are generally 10 to 15% lower than these equivalent figures, according to the size of field and the amount of land devoted to headlands, ditches, etc.

*) Received for publication: January 26, 1956.

CONTROL

It has been found in advisory work that severe attack by *Ditylenchus dipsaci* often occurred on oat crops which also had symptoms of manganese deficiency. The experimental correction of such a deficiency by watering with 1% solution of $MnSO_4$ resulted in improved manganese status of the oat tissues and promoted better growth of the crop, but did not reduce the severity of attack by the eelworm.

Field experiments in 1949 were devoted to an unsuccessful attempt to control tulip-root by the injection of D.D. dichloropropane-dichloropropylene mixture) as for the control of other eelworm diseases. There was no measurable effect of injections at 400 lb. per acre (449 Kg. per hectare). This failure to reduce the attack of *Ditylenchus dipsaci* or to improve the yield by treatment of the soil mass suggested that control might be more successful if applied at the soil surface, where the eelworm is most active. Treatments were accordingly designed to spray control material evenly over the surface, followed by some mechanical device which would work the added material into the top layer of soil. D.D. applied in this manner in 1951 at the rate of 380 lb. per acre (427 Kg. per hectare) raised the grain yield by 1.7 cwt. per acre (96.5 Kg. per hectare) over the control yield of 2.8 cwt. (159 Kg.). It also improved the establishment of the crop by 39%, and there was a 51% reduction in the number of plants seriously attacked by *Ditylenchus dipsaci.* Surface applications gave some degree of control where "soil mass" treatments gave none, but D.D. was quite uneconomic, as it would have cost about £ 30 per acre.

Heavy soil dressings of mercuric chloride were found by Johnson (1936) to bring some control of potato root eelworm and the writer (Grainger, 1956) found that far smaller amounts were effective when mixed intimately with the soil. Tulip-root eelworms from infested oat tissues were, however, found to remain active in 0.1% mercuric chloride solution longer than they did in water. It would therefore seem that there is no direct toxic action, but experience with mercury compounds against potato root eelworm suggested that control might still be demonstrated by such factors as increase in yield, a greater number of crop plants per unit area, and field evidence that the amount of disease had indeed diminished. The assessment of the percentage of plants having severe tulip-root was found to give a useful basis of comparison on heavily-infested land, and a severely diseased plant was defined as one having symptoms of tulip-root to such a degree that no normal fruiting head was produced.

Surface treatments with mercuric chloride solution against *Ditylenchus dipsaci* were first made in 1951, and gave good promise of economic application (table I). They were given, immediately *after* seeding, as a uniform spray of 40 to 55 gall. per acre (450 to 620 litres per hectare) of 1 in 1,000 solution (w/v) of mercuric chloride followed by one of the surface mixing devices mentioned later. Results over four years of treatment on severely-infested land are given in table I, A, and in each year there was a substantial increase in the yield of grain, better establishment of the crop and a significant decrease in the percentage of seriously diseased plants. The treatment was, moreover, economic, for the half-pound of mercuric chloride necessary for 1 acre could then be bought in bulk for about 7s., though it is about 12s. at the present time (1956), and the cost of application should not be more than about 12s. per acre. Increases in yield varied from 1.6 cwt. to 8.3 cwt. and 1 cwt. is now worth 23s. 3d.

It is not, however, very practical to raise yields between 2.8 and 7.7 cwt. per acre to 5 to 14.7 cwt. per acre, even by a treatment which is economic in that setting. Yields of 30 to 40 cwt. per acre are necessary, and a farmer would not normally attempt to treat a severely infested field. He would either use a resistant variety or avoid the growth of oats for as long as his rotation would allow. In 1953, two opportunities arose to assess the mercury treatment on oats grown in a rotation where the previous oat crop had serious tulip-root. The rotations were:

	1945	1946	1947	1948	1949	1950	1951	1952	1953
Farm I		Oats (tulip-root)	Seeds hay	Grass	Grass for drying	Grass	Grass	Grass grazed	Lea Oats
Farm II	Oats (tulip-root)	Turnips	Potatoes	Wheat	Grass for hay	Grass for hay	Grass grazed	Potatoes	Oats

Both crops in 1953 had small infestations of *Ditylenchus dipsaci*, doubtless carried over the six or seven years by susceptible weeds among the grass, or the wheat and grass in farm II. Results of the treatments are given in table I, B, and show that increases in grain yield of 5.0 and 4.8 cwt. per acre (285 and 274 Kg. per hectare) were obtained. Treatment was still economic, and here resulted in a full crop (30.6 and 44.9 cwt. per acre, or 1743 and 2553 Kg. per hectare).

Spraying without surface mixing resulted in very little change over the control (table I, C). Two and three normal treatments did not bring any greater increase in yield than did a single normal treatment (table I, D). This is in line with previous experience of mercuric

TABLE I

Summary of results from treatments with mercuric chloride solution

	Equivalent yield, cwt. per acre		No. of plants per square foot	% of plants with severe tulip root [3]
	Straw	Grain		
A. *Normal treatments* [1] *on severely infested crops*				
1951 Control	13.4	2.8	9.1	84
Treated (46.3) [1]	21.8(s)	5.0(s)	13.2	39
1952 Control	20.4	7.7	10.4	44
Treated (41.3)	35.8(S)	12.2(S)	17.1	16
1953 Control	15.8	6.4	20.3	46
Treated (55.5)	38.0(S)	14.7(S)	20.0	7
1954 Control	25.7	5.6*	24.8	48
Treated (55.2)	33.5(S)	7.2*	36.4	14
B. *Normal treatments* [1] *on lightly infested crops in rotation*				
1953				
I. Control	48.0	25.6	25.5	11.5 [3]
Treated (50.9)	52.5(N)	30.6(s)	29.6	2.0 [3]
II. Control	53.0	40.1	40.0	9.9 [3]
Treated (48.7)	51.3(N)	44.9(S)	41.0	1.9 [3]
C. *Normal surface sprays* [1] *without surface mixing*				
1952 Control	20.4	7.7	10.4	44
One spray (40.3)	20.8(N)	9.4(N)	11.6	32
Two sprays (62.3)	21.4(N)	7.5(N)	10.6	41
D. *Overdoses*				
1952 Control	20.4	7.7	10.4	44
One normal treatment [1] (41.3)	35.8(S)	12.2(S)	18.4	17
Two normal treatments (74.2)	44.1(S)	11.7(S)	16.5	18
Three normal treatments (107.5)	38.5(S)	11.4(S)	16.4	17
Five normal treatments (220)	23.7(N)	7.5(N)	11.0	40
E. *Underdose*				
1953 Control	15.8	6.4	18.2	36
Half normal treatment [2]	11.2(N)	5.8(N)	16.2	30

1) Normal surface sprays delivered the equivalent of 40/55 gall. of 1 in 1,000 mercuric chloride solution per acre, and normal treatments had surface mixing in addition. The actual equivalent number of gallons used per acre is given in brackets after each treatment. Gallons per acre × 11.23 = litres per hectare.

2) Applied as the equivalent of 46.5 gall. per acre of 1 in 2,000 mercuric chloride solution.

3) The percentage of plants showing tulip root symptoms and bearing no grain, except in series B, where the percentage is that of plants having *any* symptoms of tulip root.

(S) Comparison with the respective control by *t*-test is significant (*P* 0.05 or less). (s) Result nearly significant in comparison to the respective control (*P* about 0.1). (N) Result not significant in comparison with the respective control.

* These results are not significantly different, but were obtained by deliberately selecting plots on the best parts of the untreated area and the worst parts of the treated area.

The figures for yield in this table are used principally for comparative purposes, but actual yields in Kg. per hectare can be obtained by multiplying the amount in cwt. per acre by 57.

chloride used for the control of potato root eelworm (GRAINGER, 1956). Treatment at five times the normal rate, however, brought no improvement over the control (table I, D). A substantial underdose gave no control of the disease (table I, E), and one experiment where normal treatment was given *before* seeding gave no control whatever. The surface-treated soil was displaced by the drill coulters, and the plants therefore grew in soil which was mostly untreated. Treatments appeared to be effective only on the crop to which they were applied, and there has been no evidence of residual effect in any succeeding year.

DISCUSSION

These results show that surface-mixed applications of mercuric chloride solution can provide an economic control of tulip-root disease of oats. The control is, however, never spectacular, because of the very patchy occurrence of the trouble, and because grain increases of about 15% in lightly infested crops are not readily seen by the human eye. It is undoubtedly the best advice to grow a resistant variety on land expected to have moderate infestation, but surface treatment might well be advised in practice for high-yielding, susceptible oat varieties when their rotation comes round to land with a previous history of tulip-root.

BOOER (1944) showed that mercury compounds are reduced to elemental Hg in the soil, and later (1951) propounded the hypothesis that, since metallic Hg has no direct toxic action, the known control of soil-borne disease could be explained by the assumption that mercury vapour exerted a differential retardation on host and parasite, to disturb the relationships necessary for disease to occur. It is considered that the present results are in line with BOOER's finding and interpretation. (1) The fact that the oat strain of *Ditylenchus dipsaci* can remain alive longer in 0.1% $HgCl_2$ solution than in water rules out the possibility of direct toxic action on the eelworm. (2) The ineffectiveness of spraying without surface mixing could be explained by assuming that the compound was reduced to elemental Hg at the soil surface, where it would evaporate uselessly into the air and fail to bring about any control. (3) The fact that two and three treatments brought no increased control over a single treatment shows that performance is independent of dose within these limits, but (4) a severe overdose of five times normal brought no control. On BOOER's hypothesis, this would mean that growth of the host

was then retarded as much as that of the parasite, and disease would again be severe.

NOTE ON MACHINERY FOR APPLICATION

The apparatus used in the experiments is shown in Pl. XXVI. A prime necessity in the spray boom is to use fan-producing jets and not cone jets, which do not give uniform distribution. The fan jets must, moreover, be mounted on union fittings so that all the fans can be adjusted to one plane, at right angles to the line of travel. Each fan must meet its neighbours at soil level, and the height of the boom must be carefully adjusted to ensure this.

The mixing device shown in Pl. XXVI is a small rotary harrow in which the star-shaped castings revolve freely on a central axis. Small seed harrows and square-link chain harrows have also been found satisfactory but should apparently not be more than 2 feet deep from front to back. It would appear that the aim of surface mixing is to cover as much of the mercury solution with as thin a layer of soil as can be managed, and with as little disturbance of the ground as possible.

The writer wishes to acknowledge gratefully the help of members of his staff who have helped in the investigation.

SUMMARY

Tulip-root of oats, caused by the eelworm *Ditylenchus dipsaci,* has been controlled satisfactorily by surface treatment with 40 to 50 gallons per acre (450 to 653 Kg. per hectare) of 1 in 1,000 mercuric chloride solution. This was applied from a suitable fan-jet sprayer followed by a surface-mixing device, several forms of which are mentioned. The treatment was fully economic and resulted in a full yield when given to lightly infested oats grown in a rotation having a previous history of severe infestation. Surface treatments on severely infested fields gave increases in yield but not a full crop. Treatments were given immediately *after* sowing the oats; increased rates of application brought no enhanced control, whilst treatment with half the volume of liquid was ineffective.

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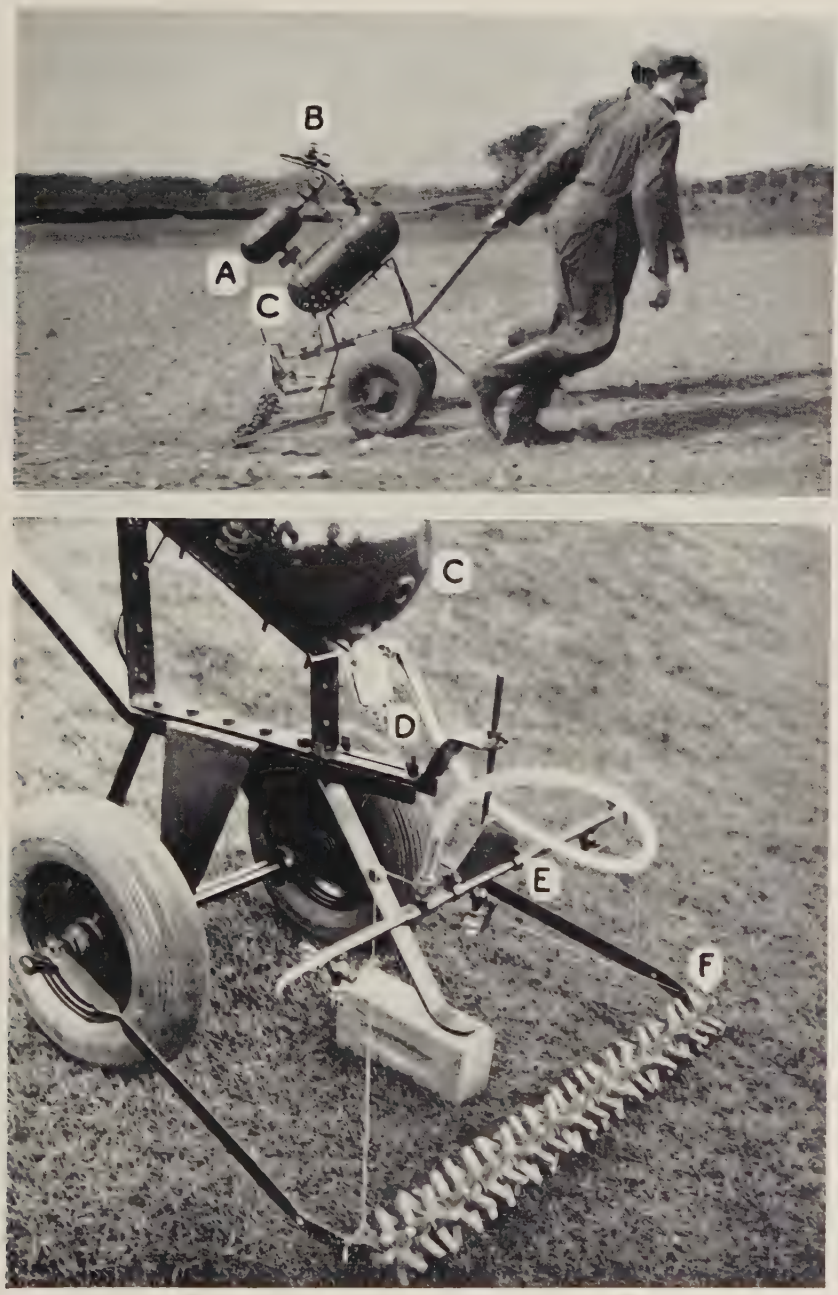

The experimental surface treating device — in use on stony ground (above), and in detail (below). A, high pressure air cylinder; B, reducing valve; C, low pressure air cylinder and liquid container; D, filter; E, spray boom; F, rotary harrow for surface mixing.

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THE DEVELOPMENT OF HETERODERA ROSTOCHIENSIS AND MELOIDOGYNE INCOGNITA IN CROSS-GRAFTED SOLANACEOUS PLANTS WITH DIFFERENT SUSCEPTIBILITIES *)

BY

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It is known that plants within the same family may show marked differences in susceptibility to attack by the same nematode root-parasite. DONCASTER (1953) found that while Tomato (*Lycopersicum esculentum* Mill.) was a host of *Heterodera rostochiensis* WOLL., another Solanaceous plant, Black Nigthshade (*Solanum nigrum* L.), was only slightly susceptible to attack, no cysts being produced. Similarly *Lycopersicum peruvianum* (L.) Mill. was found by TAYLOR & CHITWOOD (1951) to be resistant to *Meloidogyne incognita* (Kof. & White, 1919) Chitwood, 1949, while Tomato is a known host. It was thought that by cross-grafting resistant and susceptible plants together it might be possible to find out whether a resistant top would confer resistance on a susceptible rootstock, or whether a susceptible scion would cause a breakdown of the resistance of a rootstock on which it was grafted.

During 1955 two experiments were carried out to investigate this possibility. In the first, larvae of *H. rostochiensis* were inoculated into the soil in which were growing cross-grafted plants of susceptible Tomato and resistant *Solanum nigrum*. In the second, larvae of *Meloidogyne incognita* were inoculated by the same method into pots of soil containing cross-grafted plants of susceptible Tomato and resistant *Lycopersicum peruvianum*.

TOMATO AND SOLANUM NIGRUM GRAFTS INOCULATED WITH HETERODERA ROSTOCHIENSIS

Seedlings of *Solanum nigrum* and Tomato 'Moneymaker' were grown in potting compost and later potted into 3½ in. pots each containing approximately 230 gm. of compost without peat. After growing for about 8 weeks plants of comparable size were grafted

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with a splice graft, tied with self-adhesive bandage and put into a moist, heated propagating frame. Tomato was grafted onto *S. nigrum*, *S. nigrum* onto Tomato, with self-grafts of both plants as controls. When the grafts had united, the plants were moved into a cooler glass-house. At this stage side-shoots were taken off the stocks, and throughout the growing-season all side-shoots were removed as they appeared. The plants were fed at weekly intervals with a compound liquid manure.

Ten days after removal from the frame each plant was inoculated with 2,000 laboratory-hatched larvae of *H. rostochiensis* in a 2 ml. water suspension, the larvae being injected with a hypodermic needle approximately 1½" below the soil surface, and 1" away from the stem. At the time of inoculation the roots were equally well developed in all of the plants. At this stage all leaves were removed from the stocks of half of the plants, those on the remaining plants being left to see if they would counteract any effect which might be produced by the scion. Eight replications were so made in each of the eight treatments. The plants were then put outside in a partly-shaded position.

The 64 plants were lifted after two time-intervals for the cysts to be counted, as the length of time required for the formation of cysts might be longer on the cross-grafted plants than on the self-grafted Tomatoes. The first lifting of 32 plants, consisting of 4 plants from each treatment, was done 11 weeks after inoculation when white cysts were visible on the roots of the Tomatoes, and the second lifting at 17 weeks which was approximately the end of the growing-season. The contents of each pot were air-dried, washed through a Fenwick-can, and the cysts counted.

At the first lifting, cysts found in the soil from pots in which the self-grafted *S. nigrum* plants were grown were thought to be due to an older infestation in the sterilized loam used for making up the compost. After the second lifting, counts were made of the cysts with viable eggs as well as of the total numbers of cysts (Table I).

Statistical analyses of the total numbers of cysts at both time-intervals and of the viable cysts on the Tomato rootstocks only, at the second lifting, showed no significant variations due to the scions. From these results it is shown that the larvae in each root-system behaved normally irrespective of either the scion, or the presence or absence of leaves on the stock, i.e. the larvae in Tomato roots producing cysts, and those in the *S. nigrum* roots failing to do so.

TABLE I

Cyst numbers per 4 pots after growing cross-grafts of Solanum nigrum and Tomato

Type of graft:	*S. nigrum* on *S. nigrum*	Tomato on *S. nigrum*	*S. nigrum* on Tomato	Tomato on Tomato	
		Cysts after 11 weeks.			
Leaves removed from stock	8	12	52	23	95
Leaves left on stock	9	11	51	50	121
	17	23	103	73	
		Cysts after 17 weeks			
Leaves removed	12	9	50	59	130
Leaves left	8	14	50	43	115
	20	23	100	102	
		'Viable Cysts' after 17 weeks			
Leaves removed	6	1	44	49	100
Leaves left	0	0	33	35	68
	6	1	77	84	

SIDE-SHOOTS

When the grafts of Tomato and *Solanum nigrum* had united it was noticed that many side-shoots growing below the graft-union appeared to be distorted. When the side-shoots were removed, a few of the distorted ones were selected, rooted in compost, and the soil in which they were growing inoculated with 2,000 larvae of *H. rostochiensis*. Cyst-counts at the end of the growing season showed that larvae in the roots of side-shoots taken from the *S. nigrum* rootstocks had not formed cysts, while many cysts were found on the roots of side-shoots from the Tomato rootstocks. The side-shoots thus behaved as normal plants.

TOMATO AND LYCOPERSICUM PERUVIANUM GRAFTS INOCULATED WITH MELOIDOGYNE INCOGNITA

A similar experiment was done using grafted plants of Tomato and *Lycopersicum peruvianum* inoculated with larvae of *Meloidogyne incognita*. The larvae were hatched in the laboratory from egg-masses taken from infested Tomato roots. When the grafts had united, 1.5 ml. of water containing approximately 435 larvae, not more than three days old, was injected into the soil near each plant. The removal of side-shoots and leaves from the stocks, and subsequent treatment, were the same as for the Tomato/ *S. nigrum* grafted plants, except that the plants were kept in the greenhouse throughout the experiment to

maintain a sufficiently high temperature for the nematodes to develop. Forty-eight plants were successfully grafted, i.e. 12 of each of the 4 graft-types. Leaves were removed from the stocks of half of the plants, making 8 treatments, each with 6 replicates.

Galls and egg-masses were found on the roots of the Tomatoes 8 weeks after inoculation and the 48 plants were then lifted for counts to be made. The plant stems were cut off at soil level and the roots were then washed carefully to remove all soil, weighed, and put into cold 5% formalin. Later the galls were cut from the roots of the plants, tied in muslin bags, and stained in hot 0.05% cotton-blue lactophenol in which they were left overnight. After clearing in lactophenol counts were made of the egg-masses.

During the counting of the egg-masses it was found that a proportion of the females was not fully developed and had not reached the egg-laying stage. Counts were then made of the females without egg-masses, giving the total number of females in each root-system, and the percentage of those with eggs.

When the counts had been completed it was found that, while all of the plants with Tomato root-systems had many females, those with *L. peruvianum* roots had none or only a very few. These were nearly all confined to 5 of the 24 plants and were scattered at random throughout the four treatments. The slight susceptibility of these plants was thought to be due to variability in the strain of seed used. Analyses were done on the remaining four treatments consisting of the plants with Tomato root-systems.

The counts of the total numbers of females found in the Tomato roots were analysed using square-root transformations. The analysis showed the effect of the resistant scion to be significant at the 0.1% level, the plants with the *L. peruvianum* scions having fewer nematodes in their roots, than had the self-grafted Tomatoes. The presence or absence of leaves on the stock and the interaction between tops and leaves were not significant. The analysis and the means of the numbers of females in the four treatments are given in tables II and III.

A second analysis, done on angular transformations of percentage of females which had produced egg-masses, showed that the effect of the scion was significant at the 1% level, a greater percentage of the females on the self-grafted Tomatoes having reached the egg-laying stage than had the females on the roots of the Tomatoes with *L. peruvianum* scions. The effect of leaves was insignificant. There was no

TABLE II

Numbers of females on grafted plants of Tomato and Lycopersicum peruvianum: totals of 6 plants

Type of graft: —	*L. peruvianum* on *L. peruvianum*	Tomato on *L. peruvianum*	*L. peruvianum* on Tomato	Tomato on Tomato	
Leaves removed from stock	33	10	1488	1774	3305
Leaves left on stock	19	7	1436	1876	3338
	52	17	2924	3650	

TABLE III

Analysis of square-roots of total numbers of females: Tomatoes only

	D.F.	Mean square	Variance Ratio	Significance
Scions	1	20.28	15.8	P. $<$ 0.1%
Leaves	1	0.04	—	Insignificant
Scions × Leaves	1	0.95	—	Insignificant
Error	20	1.28	—	

significant interaction between the scions and the leaves (Tables IV and V).

It was thought that the slower rate of development of females in the Tomatoes with *L. peruvianum* scions might be due to reduced root development in these plants compared with that of the self-grafted

TABLE IV

Percentage of females with eggs: means of 6 counts

Type of graft: —	*L. peruvianum* on Tomato	Tomato on Tomato	
Leaves removed from stock	73	86	79
Leaves left on stock	81	93	87
	77	89	

TABLE V

Analysis of angular transformations of percentage of females with eggs: Tomato roots only

	D.F.	Mean Square	Variance Ratio	Significance
Scions	1	563.6	11.1	P $<$ 1%
Leaves	1	191.0	3.8	Insignificant
Scions × Leaves	1	0.6	—	Insignificant
Error	20	50.8		

Tomatoes. An analysis, in square-root transformation, of the root-weights of plants with Tomato root-systems taken at the time of lifting showed that this was not so, there being no significant variation between the two groups of plants. (Table VI).

TABLE VI

Root weights, in grms., of grafted plants of Tomato and L. peruvianum; Tomato root-systems only: totals of 6 plants

Type of graft:	*L. peruvianum* on Tomato	Tomato on Tomato	
Leaves removed from stock	192	169	361
Leaves left on stock	196	198	394
	388	367	

CONCLUSION

In the first experiment it is shown that when plants of Tomato and *Solanum nigrum* are cross-grafted the development of larvae of *H. rostochiensis* in their root-systems is not affected, there apparently being no transmission of susceptibility from the Tomato scion to the resistant *S. nigrum* rootstock, and the resistance of the *S. nigrum* scion having no effect on the rootstock of the susceptible Tomato. The second experiment indicates that while a Tomato scion will not reduce the resistance of *L. peruvianum* to an attack by larvae of *M. incognita,* a scion of *L. peruvianum* will considerably reduce the susceptibility of a Tomato root-system to a similar attack. This was proved by the decreased numbers, and the retarded development of the females found in the roots of Tomatoes grafted with *L. peruvianum* scions.

Comparing the two experiments: in neither case is the susceptible factor transferable from the scion to the resistant root-system in sufficient quantity to affect larval development within the roots, but in the case of *M. incognita* in cross-grafts of *L. peruvianum* with Tomato, an inhibiting factor is apparently transmissible downwards from the resistant scion of *L. peruvianum* to the susceptible Tomato rootstock. Thus it may be concluded that the effects on their nematode parasites of grafting resistant and susceptible plants together will vary with either or both the types of plants used, and with the species of nematode.

The work on grafted plants inoculated with *Heterodera rostochiensis* was proposed by Prof. B. G. Peters. I wish to thank Mr Brian Church for his advice on the analyses of the data.

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SUMMARY

Two experiments have been done to find out whether cross-grafting resistant and susceptible Solanaceous plants would affect the development of nematode parasites within their roots. Firstly, cross-grafted plants of susceptible Tomato (*Lycopersicum esculentum* MILL.) and resistant Black Nightshade (*Solanum nigrum* L.) were inoculated with larvae of *Heterodera rostochiensis* WOLL. In these plants the larvae developed normally irrespective of the scion, cysts being found on plants with a tomato root-system and none on those with a *S. nigrum* root-system. Secondly, larvae of *Meloidogyne incognita* (Kof. & White) Chitwood, 1949, were inoculated into soil around the roots of cross-grafted plants of susceptible Tomato and resistant *Lycopersicum peruvianum* (L.) MILL. Very few female nematodes were found in the plants with *L. peruvianum* root-systems. In plants with Tomato root-systems many females were found, but the total numbers and the percentage of those with eggs were significantly lower on those with *L. peruvianum* scions than on the self-grafted Tomatoes. Thus, while there was apparently no transference of susceptibility from the Tomato scion to the resistant *L. peruvianum* rootstock, a scion of *L. peruvianum* decreased the susceptibility of a Tomato rootstock to attack by larvae of *M. incognita*.

ZUSAMMENFASSUNG

Zwei Versuche wurden durchgeführt, um festzustellen, ob kreuzweise gepfropfte resistente und anfällige Solanaceen-Pflanzen die Entwicklung von Nematoden innerhalb der Wurzeln beeinflussen. Zuerst wurden Larven von *Heterodera rostochiensis* auf kreuzweise gepfropfte Pflanzen von anfälliger Tomate (*Lycopersicon esculentum* MILL.) und resistentem Nachtschatten (*Solanum nigrum* L.) übertragen. In diesen Pflanzen entwickelten sich die Larven normal ohne Rücksicht auf das Pfropfreis. Cysten wurden an Pflanzen mit einer Tomate als Unterlage gefunden, aber keine Cysten an Pflanzen mit Nachtschatten als Unterlage.

Weiterhin wurden Larven von *Meloidogyne incognita* um die Wurzeln von kreuzweise gepfropften Pflanzen einer anfälligen Tomate und einer resistenten *Lycopersicon peruvianum* (L.) MILL. in den Boden gebracht. Sehr wenige weibliche Nematoden wurden an den Pflanzen mit *L. peruvianum* als Unterlage gefunden. Bei Pflanzen mit Tomaten als Unterlage fanden sich viele Weibchen, aber die Gesamtzahl und der Prozentsatz von ihnen mit Eiern war beträchtlich niedriger an Pflanzen mit *L. peruvianum*-Pfropfreisern als an selbstgepfropften Tomaten. Während also anscheinend keine Übertragung der Anfälligkeit von Tomaten-Pfropfreis auf die resistente *L. peruvianum*-Unterlage eintritt, verminderte ein Pfropfreis von *L. peruvianum*, die Anfälligkeit einer Tomaten-Unterlage gegenüber dem Angriff durch Larven von *M. incognita*.

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THE BREAKDOWN OF POTATO-ROOT DIFFUSATE IN SOIL *)

BY

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TRIFFIT (1930) showed that larvae within the cysts of *Heterodera rostochiensis* are stimulated to hatch by a substance produced by the growing roots of potato plants. Further research by later workers confirmed her findings and new techniques were developed for the study of this phenomenon. Attempts have been made to utilize this response as a method of control and a series of papers by TODD and his collaborators (in various combinations 1949) record an attempt to elucidate the chemical nature of this substance. As this work is still continuing, it was considered that an investigation into the persistence of root diffusate in soil would be opportune. The experiments herein described are concerned with this latter problem.

TECHNIQUE

The potato root diffusate used in these experiments was obtained from potato plants growing in a 3 : 1 mixture of loam and sand. The leaching technique previously described by the author (FENWICK 1949) was modified; small volumes of water were added repeatedly to each pot until saturation was reached, when a further 50 ml. was added and the resulting leachings collected. Provided the plants were actively growing at the time of leaching, a consistently better diffusate was produced. A diffusate of L.A. value [1]) (FENWICK 1952) greater than 3.0 was usually obtained and three weeks treatment with this resulted in about 90 % larval emergence. The diffusate used for a single set of soil treatments was derived from one bulk stock which had been previously tested and shown to be of high activity.

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1) An L. A. value is the logarithm of the concentration of the active factor (expressed in arbitrary units) in any given sample of diffusate and is proportional to its activity.

A standard method of treatment was used in all experiments. The soil was first passed through a half inch mesh sieve, then coned and quartered four times to ensure thorough mixing. Standard volumes of soil measured in a suitably sized cylindrical tin were placed in 3 in. flower pots, the drainage holes of which had been covered by waxed perforated gauze. The pots were randomised, slowly flooded with 200 ml. of potato root diffusate and allowed to drain. Preliminary experiments in which the volume of diffusate draining through each pot was measured, indicated that the volume of diffusate held per pot was constant to within less than 10 %. After leaching, the pots were stored in a cool room where the temperature fluctuated between 12-18° C. To minimise loss of fluid by evaporation, each pot was covered by a loosely fitting paper cap.

Diffusate was recovered from the pots by leaching with water, the leachings being collected and made up to a known volume, then put through the pot three times to ensure equilibrium with the soil solution. The soil was normally discarded after leaching. One set of leachings was collected immediately drainage had ceased—usually about 30 minutes after addition of diffusate. Assay of this preliminary sample provided a reference point with which later leachings could be compared.

Leachates obtained from each pot were stored separately in medicine bottles in a refrigerator and, when the experiment was complete, were assayed by the method previously described (Fenwick 1949). For this purpose batches of 100 potato root eelworm cysts or alternatively weighed batches of about 250 cysts were used. After randomisation of the batches, the cysts were soaked in tap water for 14 days, after which the water was removed and replaced by an appropriate sample of leachate—each leachate was tested in five fold replication. Larvae were removed and the leachate renewed weekly; hatching was continued for 3 weeks, the larvae being accumulated during this time; enumeration was accomplished by means of a dilution technique. The data presented in this paper are therefore based on larval emergence from 5 batches of at least 100 cysts over a period of 3 weeks for each pot. The hatch in a given sample of leachate was interpolated on to a standard assay curve derived at the same time under identical conditions, the L.A. value being read off on the abscissa.

PROCEDURE AND RESULTS

Breakdown in loam

A preliminary experiment was carried out to obtain some rough estimate of root diffusate persistence in soil. Three-inch pots were filled with infested Rothamsted soil (heavy clay, loam). Each pot was flooded with 200 ml. of active potato root diffusate, allowed to drain and then stored until required. The first leaching was done immediately and thereafter at 1, 2, 4, 8, 16, 32 and 64 days: pots were in four fold replication. Three similar pots were leached in an identical manner without previous treatment with potato root diffusate and the leachates from these pots showed no activity. Leachates from the treated pots were diluted to ¼ with tap water and tested for activity against a series of dilutions of a stock sample of root diffusate. The results of this test are given in Table I, together with corresponding data for larval emergence in the standard dilution series. The results

TABLE I

Larval emergence in leachates from loam after treatment with potato-root diffusate, with an assay series for comparison

Data equivalent to hatch over 3 weeks.

Leachate Tests

Time of leaching after treatment, in days		0	1	2	5	8	16	32	64
Pot No.	1	125	117	100	40	21	16	5	6
	2	114	143	73	52	23	19	7	2
	3	120	136	88	36	17	17	6	8
	4	134	57	111	47	23	18	8	1
Total		493	453	372	175	84	70	26	17

R.D. Dilution Tests. Assay count

Dilution	1/1	1/4	1/16	1/64	1/256	1/1024	1/4096	Water
	231	230	141	111	77	26	9	9
	207	156	151	150	67	12	7	12
	254	211	166	109	55	20	10	15
	282	182	174	91	57	13	7	16
Total	974	779	632	461	256	71	33	52

of the experiment are given in detail to show the method of assay for leachates. The data for emergence in leachates represent the mean calculated hatch per 1 cyst in each leachate but are of course the results of dilution counts. A graph of emergence in the dilution series is given in Fig. 1 and will be seen to be linear for dilutions from

1/1 to 1/1024, the line being drawn by eye; the L.A. of the stock diffusate being approximately 3.1. The totals in Table 1 for larval emergence in the leachates, and which are equal in weight to those for emergence in the diffusate dilutions are interpolated on this line and correspond to the points A, B, C, D, E and F: the values for 32 and 64 day leachates were considered too low to plot on the line, and are regarded as indicative of negligible hatching activity. The L.A. values interpolated on Fig. 1 are now plotted in Fig. 2 against

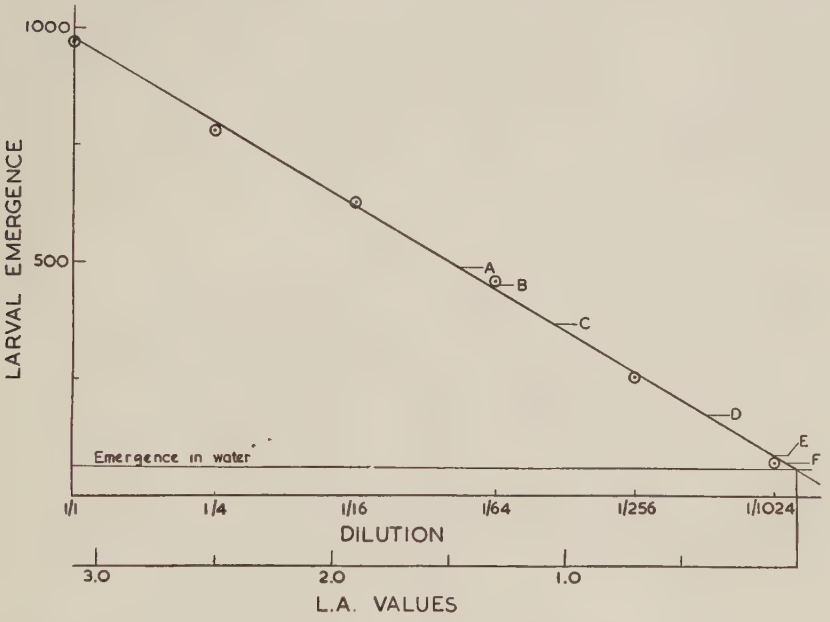

Fig. 1. Dilution curve for Root Diffusate Standard with emergence values interpolated. L. A. = Log dilution. Stock diffusate = 1/1, i.c. undiluted, with a corresponding L.A. value of approx 3.1.

time to show the form of the breakdown curve. This graph shows that there has been a rapid breakdown of the diffusate in the soil. The leachate after five days has an L.A. value of 0.4 compared with an initial value of 1.51—a fall of 1.1 corresponding to a loss of over 90 % in concentration of the active factor. By 16 days, the L.A. had fallen to 0.06—a fall of 1.45—concentration has fallen to about 1/30 of its original value—a loss of activity of about 96 %.

As the soil used in this experiment was infested with cysts of *Heterodera rostochiensis* it was thought that an investigation into the effect of root diffusate treatment on the cyst population would

be interesting. Cysts from untreated soil contained 116 eggs per cyst. After leaching the soils were dried, the cysts extracted and egg contents estimated. The results up to 64 days are given in Table II, the figures being the mean egg content per cyst over each treatment. It

Fig. 2. Breakdown of Root Diffusate in loam.

TABLE II

The egg contents of cysts recovered from soil treated with potato root diffusate

		Time in days after treatment					
	Untreated	2	4	8	16	32	64
Eggs per cyst	116	108	84	95	95	89	92
% of original content	100	93	72	82	82	77	79

is apparent that little larval emergence occurred as a result of treatment; there is no evidence of any trend in the results and it appears that a single saturation of the soil under test with active potato root diffusate has little effect on the egg content of the cysts within it.

Repetition of this experiment gave a similar result.

Breakdown in different soil components

To investigate the factors affecting the rate of breakdown of diffusate in soil, a preliminary experiment involving four separate soil components—horticultural peat, clay, sand, and gravel—was set up.

The L.A. values of the leachates obtained immediately after application of root diffusate were very variable however, possibly due to the different moisture holding capacities of the components, and no conclusions about the rates of diffusate breakdown could be drawn. A repetition gave similar results.

To overcome this difficulty the experiment was repeated, but the pots were flooded with water before treatment and allowed to drain; 50 ml. of very active root diffusate (L.A. about 3.6) was then added to each pot. The leachates collected from these pots were made up to 100 ml. and assayed in the ordinary way; leachings were taken at 2, 4, 6, 9, 12, 18, 27, 41 and 61 days; replication was five fold resulting in a total of 200 pots. The mean L.A. value for leachates from each soil component at different times after treatment with root diffusate are given in Table III. The initial L.A. value of leachates from peat, clay

TABLE III

L.A. values of leachates collected from peat, clay sand and gravel at different times after treatment with 50 ml. of root diffusate

Time in days	Peat	Clay	Sand	Gravel
0	2.1	2.0	2.1	1.7
2	1.9	2.0	1.7	1.3
4	2.3	1.3	1.1	0.7
6	2.2	1.1	0.6	0.5
9	2.1	1.1	0.8	0.3
12	2.0	0.8	0.6	0.3
18	1.9	0.6	0.6	0.3
27	1.7	0.5	0.4	0.4
41	1.2	0.1	0.3	0.2
61	1.1	0.1	0.2	0.2

and sand are seen to be fairly constant (2.0-2.1) but that for gravel leachate is lower—this may be because even 50 ml. was more than the gravel could retain, or it may be merely an anomalous result. To compare the rate of breakdown for each component the decrease in L.A. value of each was plotted against time and is presented in Fig. 3. The irregular fluctuations in L.A. values for diffusates taken from peat in the early stages of the experiment must be considered to represent random errors in the assay and indicate that breakdown in peat in up to 9 days is insignificant; thereafter a slow breakdown occurs up to 60 days. In the other components the fall in L.A. values was more rapid. Up to 18 days it appears to be most rapid in the

case of sand and slowest in the case of clay, gravel showing an intermediate rate: thereafter breakdown in gravel slows up very considerably and that in clay accelerates; at 61 days peat leachate had only fallen by 1.0 L.A. units, gravel by 1.4, and sand and clay by 1.9. Caution is necessary in interpreting these results especially so since the estimation

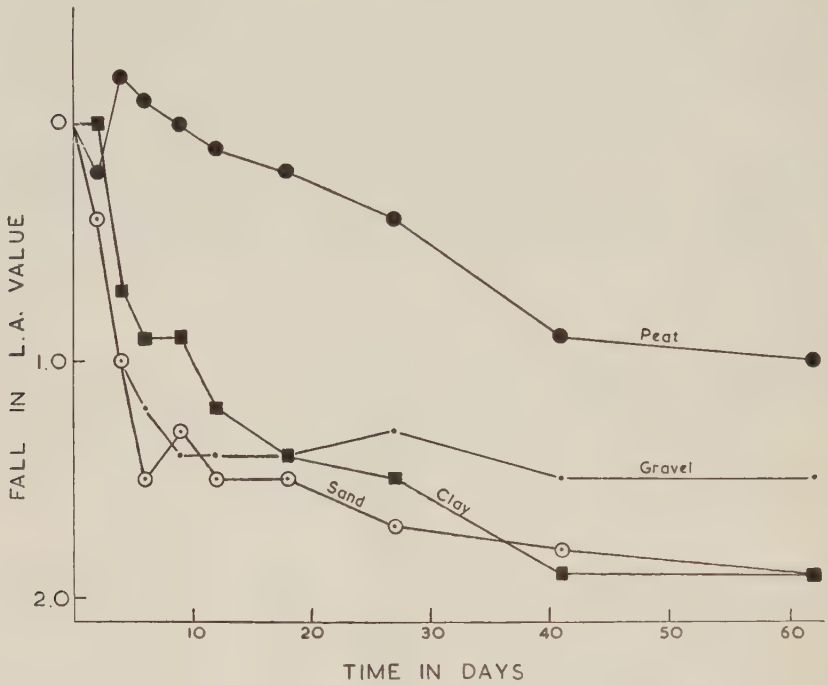

Fig. 3. Breakdown in peat, clay, sand and gravel after treatment with equal volumes of Root Diffusate.

of L.A. values below 1.0 is not precise, below L.A. 0.5 departure from linearity in the dilution curve usually occurs. The conclusion drawn from this experiment is that breakdown of diffusate in peat is much slower than in clay, gravel or sand.

REPEATED APPLICATIONS OF DIFFUSATE

Previous experiments indicate that except in the case of peat, breakdown of diffusate in soil is a comparatively rapid process. It was considered that it would be interesting to investigate the effect of repeated treatments of soil with diffusate on the breakdown rate of

the latter. Twenty five pots were filled with a mixture of 3: 1 loam and sand. These pots were flooded with root diffusate and a series of leachings at 1, 2, 4 and 8 days were taken from them. Each pot after leaching was immediately flooded again with diffusate. Fourteen days after the first flooding, all pots were again treated with potato root

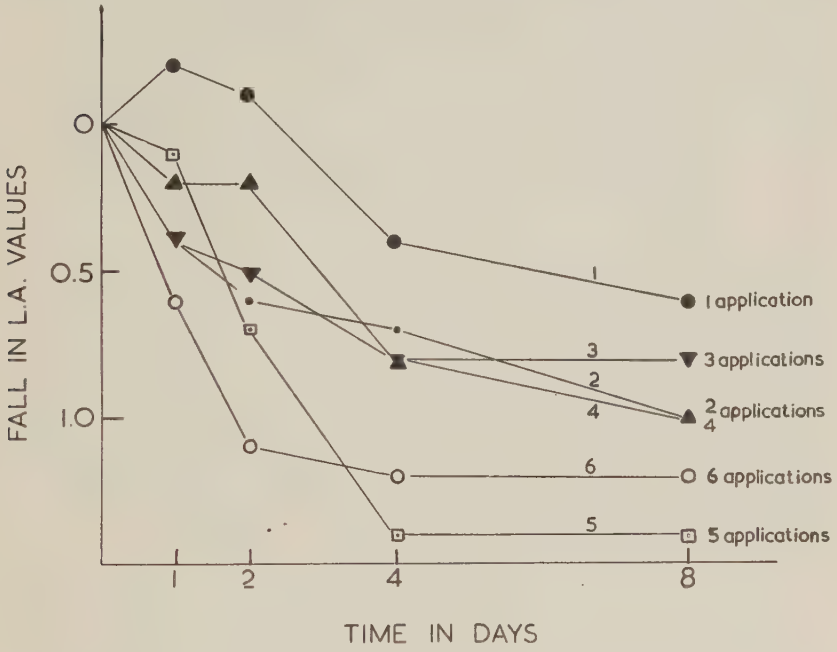

Fig. 4. Effect of repeated application of Root Diffusate on its rate of breakdown in soil.

diffusate and another set of leachings taken. Again the pots were flooded with diffusate and a third set of leachings taken. In all the treatment was repeated six times and in this way 6 sets of breakdown curves were obtained. The mean L.A. value of 5 pots for each time and each treatment are set out in Table IV and the fall in L.A. values are presented graphically in Fig. 4. There is much variability but the results suggest that the effect of repeatedly treating soil with diffusate is to accelerate the breakdown of the latter. Two further experiments have been carried out which confirm this tendency.

An experiment was carried out to investigate this effect further. Diffusate was shaken up with soil which had received several treatments with diffusate or water. Ten pots of a 3: 1 mixture of loam

and sand, after randomising, were flooded, five with tap water and five with root diffusate. The floodings were carried out twice weekly for 6 weeks so that each pot received 12 floodings with either water

TABLE IV

L.A. values of leachates collected at different times from loam after repeated treatments with potato root diffusate

Time in days	0	1	2	4	8
1st application	1.3	1.5	1.4	0.9	0.7
2nd application	1.7	1.3	1.1	1.0	0.7
3rd application	1.5	1.1	1.0	0.7	0.7
4th application	1.3	1.1	1.1	0.5	0.3
5th application	1.6	1.5	0.9	0.2	0.2
6th application	1.4	0.8	0.3	0.2	0.2

or root diffusate. At the end of this time the soil from each of the ten pots was taken and shaken with 100 ml. of potato root diffusate, allowed to settle and the supernatant liquid poured off and filtered. The ten samples of root diffusate were then stored in an incubator at 24° C to allow breakdown to occur; samples were withdrawn periodically and transferred to a refrigerator. At the end of 30 days these samples were assayed and the breakdown curves plotted. They were no steeper than those for samples mixed with the water treated soil. Over 30 days both dropped in L.A. value by about 1.2-1.3.

EFFECT OF SOIL STERILIZATION ON DIFFUSATE BREAKDOWN

The more rapid decay of diffusate in soil repeatedly treated with diffusate may be due to the presence of organisms capable of attacking the active factor in potato root diffusate. Partial soil sterilization would modify the soil micro flora and fauna and might therefore affect the rate of root diffusate breakdown. A mixture of 3: 1 loam and sand were sterilized by raising the temperature to 85° C for 20 minutes. After cooling and mixing, 30 three inch pots were filled with the sterilized loam and the rest was spread out in enamel trays for 3 and 6 weeks exposure to the air before potting. Immediately after potting the soil was treated with diffusate and a set of leachings taken. The resultant leachates were assayed and the mean L.A. value of leachates from each set of five pots are set out in Table V. The leachates taken immediately after flooding varied considerably in activity; leachates from unsterilized soil started at L.A. 2.3 while from freshly sterilized soil their activity had fallen to L.A. 1.1; this

is in accordance with the findings of Johnson & Townsend (1949) that freshly sterilized soil inhibited the hatching activity of potato root diffusate but that this inhibitory effect declined on standing. The first leachates from soil sterilized three weeks before treatment were

TABLE V

L.A. values of leachates recovered at different times after treatment of loam with potato root diffusate at different times after sterilization

Time in days	0	1	2	4	8	16
Unsterilized soil	2.3	2.2	1.2	0.8	0.3	0.2
Freshly sterilized soil	1.1	0.8	0.8	0.6	0.5	0.4
Soil sterilized 3 weeks before	1.0	1.2	1.2	0.9	0.8	0.6
Soil sterilized 6 weeks	2.4	2.1	1.6	1.4	1.0	1.3

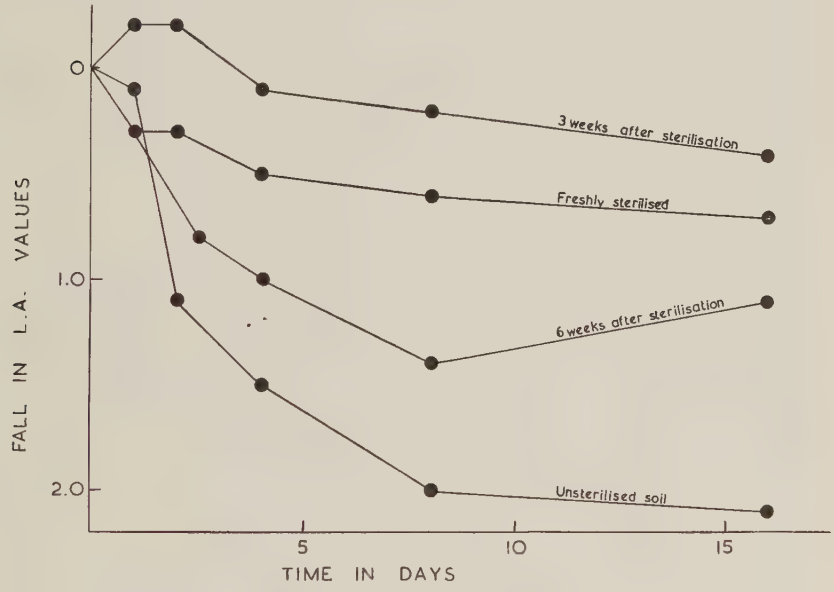

Fig. 5. Effect of soil sterilisation on the breakdown rate of Root Diffusate.

also of low activity but those from soil sterilized 6 weeks before treatment were fully active. The fall in L.A. values are plotted in Fig. 5. Little or no breakdown occurred in the freshly sterilized soil nor yet in the soil sterilized three weeks before flooding. In soil sterilized 6 weeks before flooding breakdown was more rapid but not so marked as in the unsterilized soil. In 16 days the latter had fallen in L.A. value by more than 2.0 corresponding to less than 1% persistence of the active principle but the 6 weeks sterilized soil had only

fallen by L.A. 1.1 corresponding to persistence of about 10% of the active principle.

In a repetition of this experiment a similar result was obtained.

DISCUSSION

The above results show that breakdown of diffusate in soil is fairly rapid. This would explain why a single application of diffusate to a medium loam soil had little effect on the egg content of the cyst population. The use of diffusate or of its active principle would therefore appear to be an impractical approach to the problem of control of potato root eelworm.

The results of the experiment on soil components, repeated application and sterilized soil together throw some light on the mechanism of breakdown. In surveying the results three phenomena are apparent —breakdown in horticultural peat is slower than in sand, gravel or clay, repeated applications of root diffusate result in accelerated breakdown and the application of diffusate to steam sterilized soil results in retarded breakdown. It is suggested that these results are consistent with the presence in soil of some organism capable of utilizing the active factor as a substrate and in so doing breaking it down.

In the light of this hypothesis the data on repeated applications become comprehensible. Following the first application, diffusate is broken down by soil organisms which multiply and in so doing, render possible a faster breakdown following the second and subsequent applications. The use of an automatic soil percolator as described by Lees (1947) might furnish valuable additional data on this point and also render possible the isolation of the responsible organism. The data on breakdown in sterilized soil furnishes corroboratory evidence that soil organisms play a part in breaking down diffusate. In sterilized soil the active principle breaks down very slowly. It is not unreasonable to suppose that this might be connected with the changes in soil fauna resulting from sterilization. In horticultural peat, which is known to inhibit bacterial action, the breakdown is slower than in other components. It is however interesting that when root diffusate is shaken with soil which has been repeatedly treated with diffusates the breakdown of the diffusate at 24° C after coarse filtration is no faster than that of a similar sample shaken with ordinary soil; it is possible however that a solution of root diffusate which provides a different environment from that found in soil is not the most suitable medium for the microbiological activity.

SUMMARY

Experiments on the stability of potato-root diffusate in soil are described. 90% of the active principle was lost in four days in a medium grade loam. Comparative tests with soil components showed that breakdown was most rapid in sand and gravel and slowest in peat. When repeated applications of diffusate were made to a loam, successive breakdown curves were each steeper than the previous, suggesting the presence of a microorganism capable of utilising the active principle as a substrate. This was confirmed by results with sterilised loam. Newly sterilised loam inhibited the action of root diffusate but, on being allowed to stand for about six weeks, was no longer inhibitory. When diffusate was applied to loam that has been allowed to stand the breakdown was slower than in untreated soil. These results in conjunction with those in peat, tend to confirm the theory of breakdown by micro organisms.

ZUSAMMENFASSUNG

Versuche über die Stabilität des Kartoffelwurzeldiffusats im Boden werden beschrieben. 90% des aktiven Stoffs gingen innerhalb von 4 Tagen verloren, nach 16 Tagen betrug der Verlust etwa 96%. Vergleichende Versuche mit mehreren Böden (Torf, Lehm, Sand und Kies) zeigten, dass der Wirkungsabfall bei Sand und Kies sehr schnell, bei Torf aber langsamer als bei Lehm erfolgte. Bei wiederholter Anwendung von Wurzeldiffusaten wurde der Wirkungsabfall des aktiven Stoffs bei jeder der nachfolgenden Gaben beschleunigt. Aus diesem Befund wird auf das Vorhandensein eines Mikroorganismus geschlossen, der den aktiven Stoff als Nahrungsquelle benutzen kann. Die gleiche Folgerung ergibt sich aus dem schwächeren Wirkungsabfall des Diffusats in dem mikrobiologisch wenig aktiven Torf gegenüber Lehm, Sand und Kies. In frischem und im 3 Wochen vorher sterilisiertem Boden wurde die Wirkung des Wurzeldiffusats gehemmt; wenn das Diffusat aber zu sterilisiertem Lehm angewandt wurde, der 6 Wochen lang unter Luftzutritt gelagert hatte, war die Wirkung der Aktivierungssubstanz grösser, edoch nicht so gross wie in nicht sterilisiertem Boden.

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COLLECTION OF CYSTS OF HETERODERA MAJOR AND ESTIMATION OF THE CYST CONTENT *)

BY

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The most well-known method for collection of cysts is the use of the Fenwick-can (GOODEY 1951). The principle is that dry cysts float in water, whereas the heavier soil particles sink. In the case of potato and beet eelworm no trouble in this respect is reported, but some of the full cysts of the oat eelworm (*Heterodera major*) will sink in water. Only a few cysts sink, but these are always full cysts, and the average content of eggs and larvae is usually above 200. HESLING noticed the 'heavy' cysts in experiments at Rothamsted and he suggested to dry the float from the Fenwick-can prior to its refloating (HESLING 1956). A similar modification of the Fenwick method has been used in the writers experiments in 1954 and 1955.

Cyst collection is the first step in determining the infectivity of the soil. The next is to determine the number of eggs and larvae inside the cysts. The most accurate method for determination of cyst content is probably the one developed by BIJLOO (1954). The method described below is a cheaper and more simple method.

Collection of cysts. The number of non-floating cysts was high in the autumn of 1954. In some samples the heavy cysts contained up to 25% of the total number of eggs and larvae. In 1955 such cysts were noticed too, and the formation of heavy cysts seems to be quite normal for the species. These heavy cysts can be collected, if a Fenwick-can is used, but a slight modification of the method is necessary.

The first step is to dry the soil at a high temperature. The number of heavy cysts is considerably lower in soil dried at a temperature of 110-120° C than in air-dry soil or in soil dried at 70-80°. The next is use of much water. When all soil particles have been washed from the upper sieve it is useful to put the end of the water hose into the

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Fenwick-can and run a strong current of water in the can. This will stir the heavy particles in the lower part and bring any cysts to the top.

According to the method usually applied the float from the Fenwick-can is collected on a sieve and transferred to a flask. The flask is filled with water, and the cysts float to the top. In the case of *Heterodera major* water must be replaced by another liquid, e.g. a solution of $MgSO_4$ with a specific gravity of 1.20. Most of the cysts will float in this liquid, and the rest will swim at different levels in the flask. The method is useful if the loss of a single or a few swimming cysts is unimportant. If a total recovery of all cysts is required, another modification is possible.

The cysts and debris which float in the Fenwick-can may be dried and thereafter cleaned by rolling as described below. The rolling material is transferred to a funnel which is connected to a plastic flask by a rubber tube (Pl. XXVII, fig. 1). The flask and the funnel are filled with dissolved $MgSO_4$ with a specific gravity of 1.15-1.20 and dry cysts and debris are spread on the surface of the liquid. Heavy particles sink into the flask, and after 4-5 minutes the funnel and the tube are removed. All cysts are in the liquid in the funnel and tube, and they are collected on a filter. No cysts sink into the plastic flask and consequently it is not necessary to clean the flask between two samples.

Removal of long or flat particles. The debris contains many long or flat particles, much of which can be removed. An easy method is to spread cysts and debris on a rolling paper or rubber belt (Fig. 1). Cysts and rounded-shaped particles roll down the belt and are collected, whereas long particles remain stationary on the belt and are transferred to the top and discarded. In many samples 80-90% of the debris is removed by treatment on the belt, and the final collection of the cysts is easier.

Machines with a rolling belt are now in use in several places where work with *Heterodera* species is carried out. In most cases the apparatus is home-made and each worker has made it in his own way. However, in all cases the machine consists of a rolling belt driven by a small electromotor.

In the machine used in the writer's experiments, the belt is made of ordinary typewriting paper. The length of the belt is 45 cm, and it is driven by a 40 watt electromotor. The speed of the belt is 3.4

Fig. 1. The floating system described in the text.

Fig. 2. Filling of a slide from a plastic flask.

and treatment for 5 minutes does not give a higher larval count.

After shaking for 40 seconds a modified Mc Master slide is filled with the suspension. This is very easily done by gentle pressure on the walls of the plastic flask (Pl. XXVII, fig. 2).

The method was used in routine work in 1955, and a calculation from 147 soil samples will show the reliability of the method (Table I). A suspension of crushed cysts was made from each soil sample,

TABLE I

Variation in larval count, when a modified Mc Master slide is filled from a plastic flask.

Suspensions with an average number of eggs and larvae	Number of suspensions (soil samples)	Number of eggs and larvae	Error of each subsample	Error of each suspension
From 50 to 75	38	60.7	10.0	5.8
From 75 to 100	39	89.2	9.3	5.4
From 100 to 125	44	111.8	12.0	6.9
From 125 to 175	26	141.5	12.6	7.3

and eggs and larvae were counted in 3 subsamples of one ml. The error of each subsample is calculated by the formula $\pm \sqrt{\frac{S\,(x-\bar{x})^2}{(n-1)\,(p-1)}}$, where n is the number of subsamples, e.g. 3, and p is the number of soil samples. The error of each subsample is about 11, and, as the number of subsamples was 3, the error of the mean is about 6.5.

SUMMARY

A number of full cysts of *Heterodera major* will not float, but sink in water. It is suggested to use a solution of $MgSO_4$, when cysts are collected from soil samples. A cheap method for larval count is described.

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THE RENIFORM NEMATODE IN THE GOLD COAST *)

BY

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The reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira 1940) was first described as an obligate parasite on the roots of many plants in Hawaii, and a list of 65 hosts was given by LINFORD & YAP (1940). Subsequent records from America (SMITH & TAYLOR 1941, STEINER 1947, 1949) added a further 5 hosts. MARTIN (1955) reported the occurrence of this nematode in Rhodesia on 3 hosts, 2 of which were new records.

During 1955 the writer observed the reniform nematode on a number of plants in and around Accra, the capital of the Gold Coast, West Africa, and a host list including 8 new records was compiled (PEACOCK 1956). The present paper gives a fuller account of the occurrence and biology of this nematode under Gold Coast conditions.

LOCALITY AND OCCURRENCE

(1) Achimota. University College campus. 7 miles from Accra. Light infestation on Avocado seedlings. Examination of weeds and plants in the vicinity showed Barbados Flower-fence (Poinciana pulcherrima L.) to be the only other plant infested.

(2) Pokoase. Agricultural Station 15 miles from Accra. Heavy infestation of two common weeds (Amaranthus spinosus L., Synedrella nodiflora Gaertn.) overgrowing the site of a long-demolished resthouse. The area of infestation coincided with the shade area of a large Cassia tree.

(3) Accra. Market garden. Carrot (Daucus carota L.), Tomato (Lycopersicon esculentum Mill.), Cotton (Gossypium spp.) and Kidney bean (Phaseolus vulgaris L.) grown under over-head irrigation were heavily infested.

The remaining host plants recorded were infested either accidentally or by design in pot experiments using soil from the above sites.

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MEASUREMENTS

Larva: length 330-455 μ mean 397 μ. α = 20.9, β = 4.1, γ = 12.9.
Male: length 387-475 μ mean 424 μ. α = 24.8, β = 4.3, γ = 11.6.
Mature female: full length 410-655 μ, reniform part of body 310-454 μ, mean 390 μ, diameter 122-178 μ, mean 147 μ.
Egg: 72-100 μ × 33-44 μ, mean 88 × 40 μ.

Female and egg are apparently slightly smaller, the male a little longer than those described by LINFORD and OLIVEIRA, but these measurements apply to a population on soya, and there would appear to be considerable variation in size from one host plant to another.

LIFE HISTORY

Four days after sowing in *Rotylenchulus*-infested soil, roots of Soya (Glycine max Merr.) were heavily infested with larvae, many of which were swollen forms. After 7 days mature females were observed together with the gelatinous matrix, which however contained no eggs. In some instances males were seen coiled inside the matrix.

Egg-laying had commenced by 10 days after sowing, but eggs were still undifferentiated. At 11 days most females were egg-laying, and many egg-masses contained one or more males. Of a total of 80 nematodes observed at this time 6 (7.5 %) were immature, 16 (20.0 %) were mature but had not produced the matrix, 11 (13.75 %) were mature and had produced the matrix but no eggs, and 47 (58.75 %) were mature, with matrix and eggs. Of those which had produced the matrix, 17 (29.3 %) harboured males; although more than one male per female was not unusual, the most observed in one egg-mass was 3.

Fifteen days after planting, eggs contained larvae, and some larvae had hatched, and were leaving the matrix.

At 22 days all eggs were viable, the number of eggs per female ranging from 30 to 44 with a mean of 39, but numbers of empty egg-cases were also present. At this time egg-masses on tomato gave egg-counts as high as 102 with a mean of 63, and on cotton up to 94 with a mean of 66. On a local creeping cowpea egg-counts up to 88 per egg-mass were obtained.

In hatching experiments in distilled water, moulting commenced 24 hours to 4½ days after hatching, and was completed 8 to 18 days after hatching.

HOST-PARASITE RELATIONSHIP

A wide range of host susceptibility to reniform nematode attack

Rotylenchulus reniformis Linford & Oliveira. Mature female and eggs.

Fig. 1
Rotylenchulus reniformis. Juvenile female feeding on Avocado.

Fig. 2
Rotylenchulus reniformis. Mature female and eggmasses on soya: the female has been squeezed out of the root.

was observed. Highly suitable hosts were tomato, tobacco (Nicotiana tabacum L.), okro (Hibiscus esculentus L.), garden egg (Solanum melongena L. var. esculentum), sweet potato (Ipomoea batatas Lam.), soya, carrot, and the two weeds previously mentioned.

Lighter infestations occurred on rough lemon (Citrus limon Burm. f.), avocado (Persea americana Mill.), cassava (Manihot esculenta Crantz.), and cotton (Gossypium spp.).

Resistance was shown by coffee (Coffea robusta Linden), jackbean (Canavalia ensiformis D. C.), velvet bean (Stizolobium deeringianum Bort.), and red pepper (Capsicum frutescens L. var. microcarpum). On these plants limited larval invasion occurred and some development, but eggs were never produced.

No host reaction (i.e. hypertrophy or breakdown of cell structure) was noted, but some slight necrosis of the cell walls surrounding the nematode occurred.

On suitable hosts the swollen 'reniform' part of the nematode invariably remained on the outside of the root, but on cotton and jackbean individuals were observed which were completely embedded in the root cortex.

SEVERITY OF ATTACK

The reniform nematode has not, so far, been reported as causing crop failure or reduced yield; it is interesting therefore to note here the occurrence on tomato seedlings of an infestation of such magnitude that the vigour of the host plant must have been impaired. The seedlings, which were grown in root-knot infested soil, were found on examination 14 days after planting to contain both root-knot eelworm larvae and all stages of *Rotylenchulus* females, including large numbers of egg-masses with viable eggs and empty egg cases. One hundred and fifty to 200 egg-masses per plant were counted; the root systems weighed only 1.6 to 1.9 g. giving an infection rate of approx. 100 egg-masses per gram of root.

SUMMARY

The reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira) has been observed on a wide range of food crops in the Gold Coast, and can maintain itself under fallow conditions on at least two common weeds.

Measurements differ slightly from those given by Linford and Oliveira, but it is considered that the same species is involved, the differences being accounted for by development on a different host.

The life-cycle appears to be as short, or a little shorter than, LINFORD and OLIVEIRA's theoretical minimum period of 25 days.

Susceptible and resistant host species have been noted, and a particularly heavy infestation on tomato is recorded.

Acknowledgements

The writer acknowledges help and advice from Dr. J. B. GOODEY, Nematology Dept., Rothamsted, and Dr. S. A. SHER, Division of Plant Nematology, Riverside, California. The photographs were taken by Mr. and Mrs. J. M. JUDD, Department of Tsetse Control, Achimota.

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(*Nematologica*, Vol. I, 1956, no. 4)

BEITRÄGE ZUR FREILEBENDEN NEMATODENFAUNA BRASILIENS, I. ACHT NEUE NEMATODENARTEN DER ÜBERFAMILIE DORYLAIMOIDEA [1])

VON

ARWED H. MEYL

(Aus dem Zoologischen Institut der T. H. Braunschweig)

Professor Dr. Paul Buchner
zum 70. Geburtstag

In einem umfangreichen Nematodenmaterial aus verschiedenen Lebensräumen an der Küste von Brasilien stammend, fand sich eine grosse Anzahl neuer Arten, von denen acht im folgenden beschrieben seien; ihre Ökologie soll erst in einer späteren, zusammenfassenden Arbeit durch S. A. GERLACH behandelt werden.

Es sei an dieser Stelle nochmals Herrn Dr. S. A. GERLACH/Kiel für die grosszügige Überlassung der terricolen und limnischen Nematodenausbeute gedankt, die er neben vielem anderen als Frucht einjähriger Forschungs- und Sammeltätigkeit aus Brasilen, — auf Einladung der Universität São Paulo und mit finanzieller Unterstützung der Deutschen Forschungsgemeinschaft, — zurückbringen konnte.

Dorylaimus paulbuchneri n. sp. (T. 1, Abb. 1-4)

♀ L = 1, 2-1,3 mm a = 33-39 b = 4,4-4,8 c = 15-17 V = 50% (n = 10)
Typus: L = 1,23 mm a = 38,0 b = 4,5 c = 16,6 V = 49,9%
Locus typicus: Rio Nanaú, Algen von *Rhizophora*-Stelzwurzeln.
♂ L = 1,0-1,2 mm a = 32-39 b = 4,1-4,3 c = 47-51 (n = 5)
Typus: L = 1,05 mm a = 32,4 b = 4,1 c = 47,6
Locus typicus: Wie ♀. Coll. no. (♂, ♀) 5605 [2]).

Körper schlank. Cuticula glatt, ohne Ringelung oder Längsstrei-

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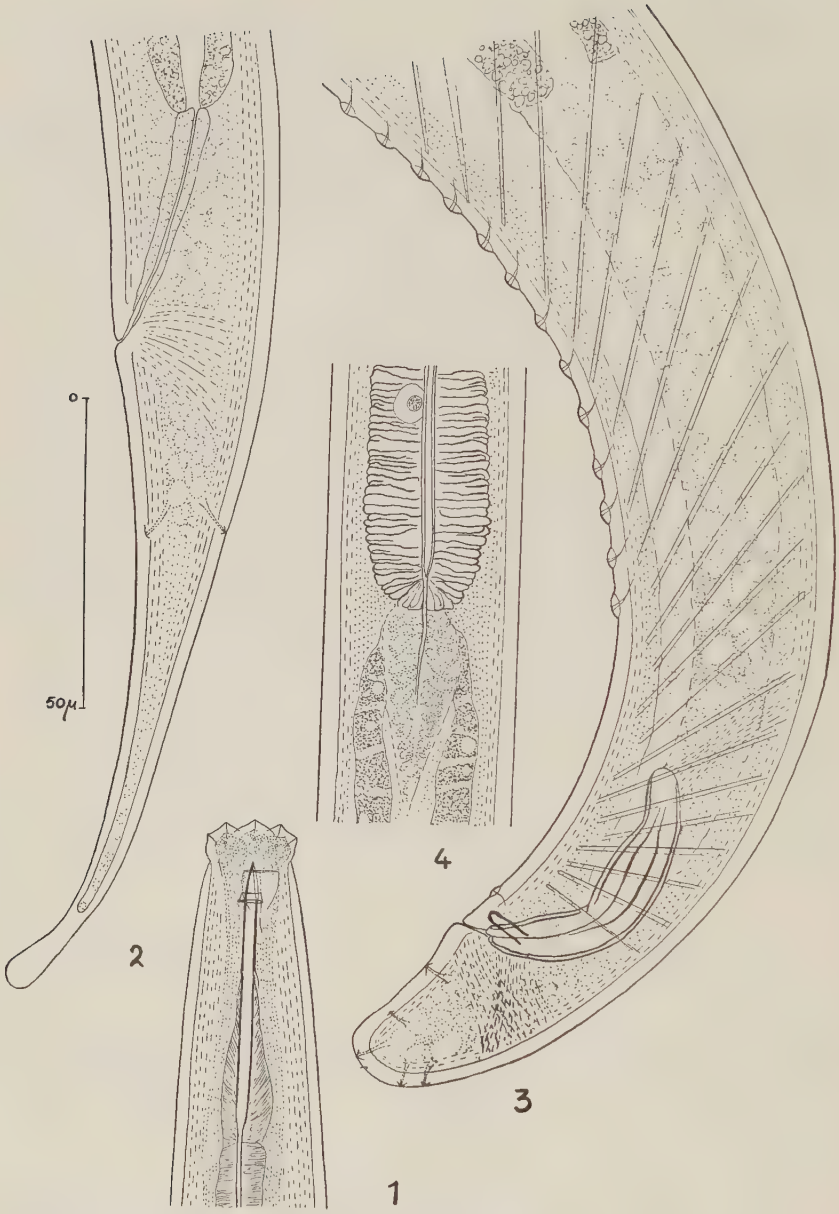
1) Eingegangen: 23 März, 1956.
2) Die Typen befinden sich in der Sammlung des Naturhistorischen Museums Braunschweig.



Tafel I. *Dorylaimus paulbuchneri* n.sp.: Abb. 1 = Kopf eines ♀, Abb. 2 = Schwanz eines ♀, Abb. 3 = Hinterkörper eines ♂, Abb. 4 = Cardiaregion.

fung. Lippen mässig stark abgesetzt, Papillen deutlich, Lippenbreite 30% des Körperdurchmessers in Cardiahöhe. Seitenorgan 40% der Lippenbreite. Stachel etwas länger als Lippenbreite und 1/8 so dick, seine Öffnung 1/3 der Stachellänge. Führungsring doppelt. Oesophagus um die Stachelverlängerung etwas anschwellend, dann bis 65% der Halslänge schmal und mässig muskulös, das letzte 1/3 ziemlich plötzlich verbreitert auf das 2 fache des schmalen Teils. Nervenring nach 35-40% der gesamter Oesophaguslänge vom Vorderende an. Im breitmuskulösen Teil besonders deutliche Drüsenkerne kurz nach dem Beginn der Verbreiterung und nach 75% deren Länge. Cardia spitzkonisch, drüsig (Abb. 4), mehr als zweimal so lang wie breit. — ♀ Gonaden symmetrisch, 16-19% der gesamten Körperlänge je Ast einnehmend, mit auf die ½ Länge zurückgeschlagenen Enden. Um die reifen Eier deutliche Spermien. Je ein Ei in G_1 und G_2, Eigrösse 65:26 µ. Vagina 43% der Körperbreite. — ♀ Schwanz gleichmässig konisch verlängert, mit zwei deutlichen Papillen; vor seinem Ende, Breite 2,5 µ, dann auf 4 µ blasenförmig anschwellend (Abb. 2). Im blasenförmigen Teil nur noch kuticulare Hülle. Rectum 1,5-1,75 Analbreiten lang, von starker Muskulatur umgeben. Deutliche Analmuskulatur. Prärectum meist 2,5 Rectumlängen lang, gut abgesetzt. ♂ Gonade typisch, Beginn bei 33% der Gesamtkörperlänge vom Vorderende an. Mit 13 Präanalorganen, die etwas ungleichmässig, etwa jeweils 1,0-1,5 Organbreiten auseinanderstehen. Beginn der Reihe 70% der Spiculalänge hinter dessen proximalem Ende. Sublaterale Papillen nicht beobachtet. Schrägmuskulatur sehr ausgeprägt. Spicula ziemlich plump, ca. 33 µ Sehnenmass lang, mit vorn quer abgestutzten, schmalen lateralen Führungsstücken. Adanales Papillenpaar vorhanden. Prärectumbeginn noch im Bereich der Präanalorgane meist in Höhe des 9. bis 10. präanalen Organs. Schwanz des ♂ konoid verlängert, ventral leicht eingebuchtet, am Ende quer abgestumpft-gerundet. Fünf postanale Papillen deutlich. Analmuskulatur gut sichtbar.

Verwandtschaft: D. aquatilis Skwarra 1921 (L = 4,0 mm, Cuticula mit Längsstreifen, Oesophagus nach 50% verbreitert, Cuticula dicker). *D. clavicaudatus* Thorne & Swanger 1936 (Lippenbreite nur 25% der Körperbreite in Cardiahöhe, Rectum nur 1 Analbreite, Stachel 1,5 Lippenbreiten lang, ♂ mit 17, sich berührenden Präanalorganen, ♂ Prärectum beginnt weiter präanal, Spicula schlanker).

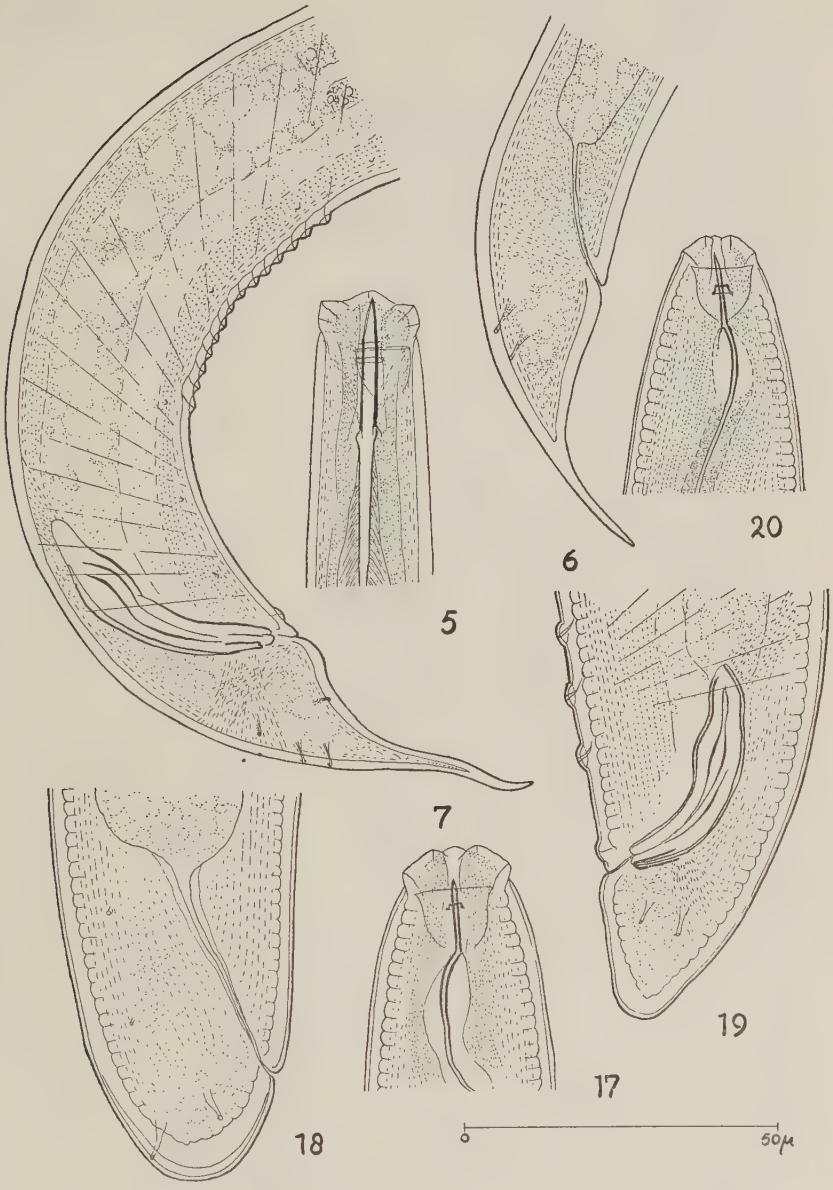
Fundort: Siehe Locus typicus!

Dorylaimus brasiliensis n. sp. (T. II, Abb. 5-7)

♀ L = 1,1-1,3 mm a = 40-53 b = 4,2-4,8 c = 27-31 V = 41-48% (n = 15)
Typus: L = 1,18 mm a = 50,5 b = 4,2 c = 30,3 V = 47,2%
Locus typicus: Santos, supralitoraler Feinsand.
♂ L = 1,0-1,2 mm a = 48-52 b = 4,3-4,8 c = 28-37 (n = 9)
Typus: L = 1,11 mm a = 50,2 b = 4,6 c = 28,5
Locus typicus: Wie ♀. Coll. no. (♂, ♀) 5606.

Körper fadenförmig schlank, nur kopf- und analwärts verschmälert, sonst zylindrisch. Cuticula glatt, ungeringelt ohne Längsstreifen. Kopfform quer abgestutzt, Lippen kaum abgesetzt, Papillen mässig deutlich. Lippenbreite 38-40% der Körperbreite in Cardiahöhe. Seitenorgan ½ Lippenbreite. Stachel 135% der Lippenbreite, $^1/_6$ so dick, seine Öffnung $^1/_3$ der Stachellänge. Führungsring doppelt, Stachelverlängerungen relativ kurz (Abb. 5). Oesophagus nach 55% seiner Länge allmählich auf die doppelte Anfansbreite verbreitert, Nervenring bei 48%, kurz vor dem Verbreiterungsbeginn. Cardia herzförmig, so lang wie breit. ♀ Gonade symmetrisch (je Ast 14-15%), Umschläge $^1/_3$ der Astlänge. Eigrösse (für ♀ L = 1,1 mm) = 97: 21 μ. Rectum 1,25 Analbreiten, Prärectum 5 Analbreiten bzw. 4-4,5 Rectumlängen. Keine sichtbare Anal- und Rectalmuskulatur. ♀ Schwanz etwas hinter seiner halben, konisch zulaufenden Länge peitschenförmig auslaufend, gestreckt oder ventral gebogen, am Ende spitz. Zwei deutliche Schwanzpapillen. — ♂ Gonade typisch, bei 36% Gesamtkörperlänge beginnend. Mit 13-14 einander berührenden Präanalorganen (insgesamt 40 μ Ausdehnung), etwa 1 Spiculumlänge präanal beginnend. Sehr zarte Subventralpapillen (ca. 7) über die Präanalorganreihe hinausreichend. Adanales Papillenpaar vorhanden. Sehr wenig markante Schrägmuskulatur. Prärectumbeginn vor der Präanalorganreihe. Spicula schlank, 26-30 μ Sehnenmass, mit sehr kleinen und schmalen, lateralen Führungsstücken. ♂ Schwanz in der Form wie beim Weibchen, mit 4 postanalen Papillen, von denen drei stärker hervortreten.

Verwandtschaft: *D. bir*ói Daday 1899 (Nur ♀ bekannt, weniger schlank, andere Kopfform, mit einem prärectalen Organ, kürzere Eier, längeres Rectum, kürzeres Prärectum). — *D. doryuris* Ditlevsen 1911 (andere Kopfform, weniger schlank, absolut grösser, Cuticula am Halsbeginn viel dicker, nur ♀ bekannt. (Ein genauer Vergleich konnte mit Nordseematerial, mir liebenswürdigerweise von S.



Tafel II. *Dorylaimus brasiliensis* n.sp.: Abb. 5 = Kopf eines ♀, Abb. 6 = Schwanz eines ♀, Abb. 7 = Hinterkörper eines ♂. — *Leptonchus multipapillatus* n.sp.: Abb. 17 = Kopf eines ♀, Abb. 18 = Schwanz eines ♀, Abb. 19 = Schwanz eines ♂. — *Leptonchus paucipapillatus* n.sp.: Abb. 20 = Kopf eines ♀.

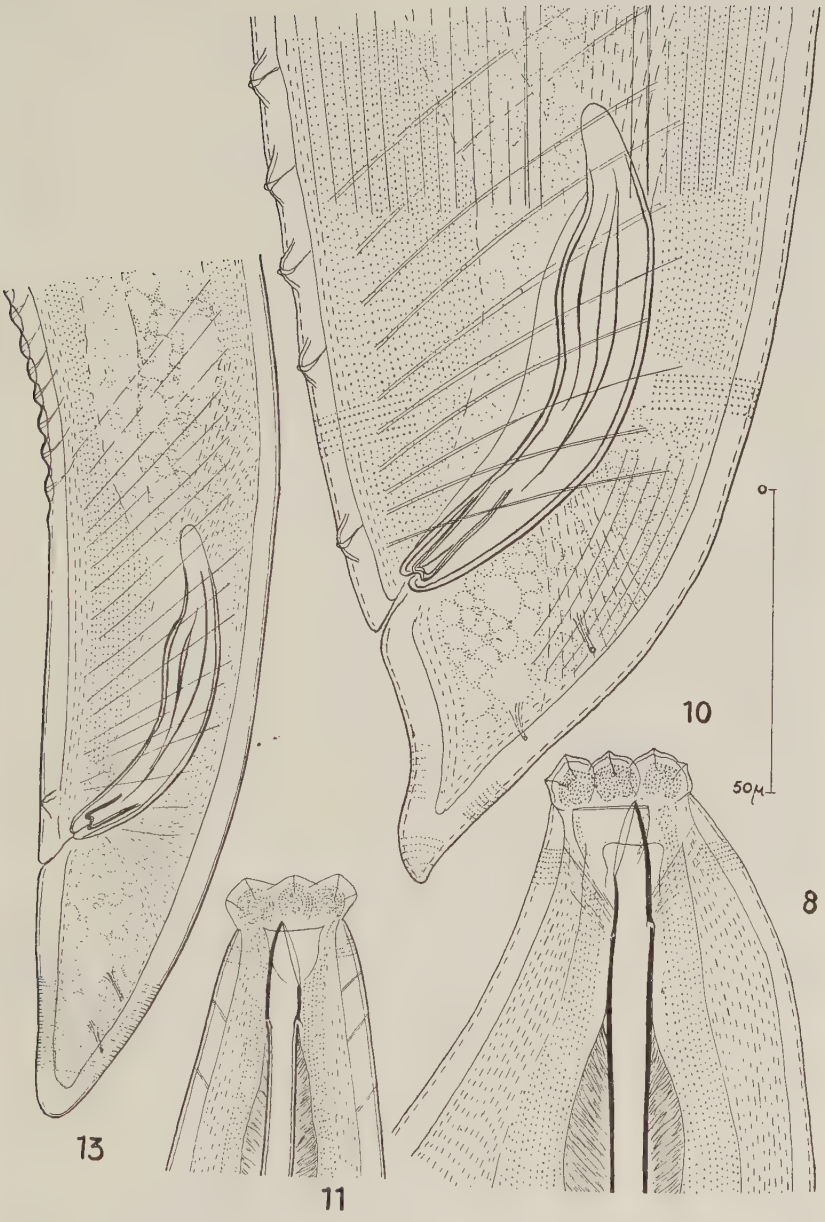
A. Gerlach zur Bearbeitung überlassen, durchgeführt werden). — *D. nigritulus* W. Schneider 1938 (nur ♀ bekannt, Schwanz länger, andere Kopfform etc.).

Fundorte: Wie locus typicus, und Solemar an der Praia grande bei Santos, Bestand von *Iresine* auf braunem Sand.

Aporcelaimus gerlachi n. sp. (T. III u. V: Abb. 8-10)

♀ L = 1,9-2,2 mm a = 18-20 b = 3,9-4,3 c = 48-52 V = 53-56% (n = 6)
Typus: L = 1,92 mm a = 20,5 b = 3,9 c = 49,3 V = 53%
Locus typicus: Cananaia, Mangrove-Sandschlick am Rio Maria Rodrigues mit Süsswassereinfluss.
♂ L = 1,7-1,9 mm, a = 22 b = 3,6-4,0 c = 65-67 (n = 5)
Typus: L = 1,74 mm a = 22,3 b = 3,6 c = 67,0
Locus typicus: Wie ♀. Coll. no. (♂, ♀) 5607.

Körper sehr plump, wurstförmig, nur am Kopf und Schwanz stark verschmälert. Cuticula in Laterallage mit 20 Längsstreifen, Seitenfeld etwa $^1/_5$ der Körperbreite, Querringelung in Punkte auflösbar, am ganzen Körper sichtbar. Cuticula dick, ungeschichtet. Lippen deutlich abgesetzt, $^1/_3$ so hoch wie breit, Papillen markant. Lippenbreite $^1/_5$ bis $^1/_6$ der Körperbreite in Cardiahöhe. Seitenorgan $^1/_2$ Lippenbreite. Stachel 20-21 μ = 1,0 Lippenbreite lang, $^1/_4$ so breit, Öffnung nicht ganz $^2/_3$ der Stachellänge. Ohne Führungsring, doch mit mässig deutlicher Hautfalte als Führung. Oesophagus bis 35-40% seiner Länge schmal (ca. Lippenbreite), dann allmählich bis auf das 3-fache der Anfangsbreite verbreitert. Nervenring bei 30%, Cardia herzförmig, fast so breit wie lang. ♀ Gonade symmetrisch, Äste 20% der Gesamtkörperlänge, Umschläge etwas weniger als die $^1/_2$ Länge der Äste erreichend. Vulva typisch, Vagina $^2/_3$ Körperbreite, Eier nicht beobachtet. ♀ Schwanz (Abb. 9) spitzkonisch, dorsal abgebogen, kurz. Cuticula bis zum Ende geringelt, ungeschichtet. Starke Analmuskulatur. Zwei deutliche Schwanzpapillen. Rectum = 1 Analbreite, Prärectum 3,5-4 Rectumlängen. — ♂ Gonade typisch, bei 40% der Gesamtkörperlänge beginnend. Mit 11-12 zäpfchenförmigen Präanalorganen, jeweils 10-14 μ voneinanderstehend. Beginn der Reihe noch im Spiculabereich, das erste Organ etwa doppelt so weit vom adanalen Papillenpaar entfernt wie vom folgenden Präanalorgan. Ausdehnung der Präanalorganreihe 117-120 μ = ca. 6 Schwanzlängen. Beginn der starken Schrägmuskulatur noch vor dem letzten Präanalorgan. Keine



Tafel III. *Aporcelaimus gerlachi* n.sp.: Abb. 8 = Kopf eines ♀, Abb. 10 = Schwanz eines ♂, — *Aporcelaimus paraconicaudatus* n.sp.: Abb. 11 = Kopf eines ♀, Abb. 13 = Hinterkörper eines ♂.

subventralen Papillen beobachtet. Spicula schlank, ohne Gubernakulum, 70 μ Sehnenmass. Laterale Führungsstücke kräftig, distal gekerbt. Schwanz wie beim ♀, ohne cuticulare Schichtung, dorsal abgebogen, mit 2 postanalen Papillen.

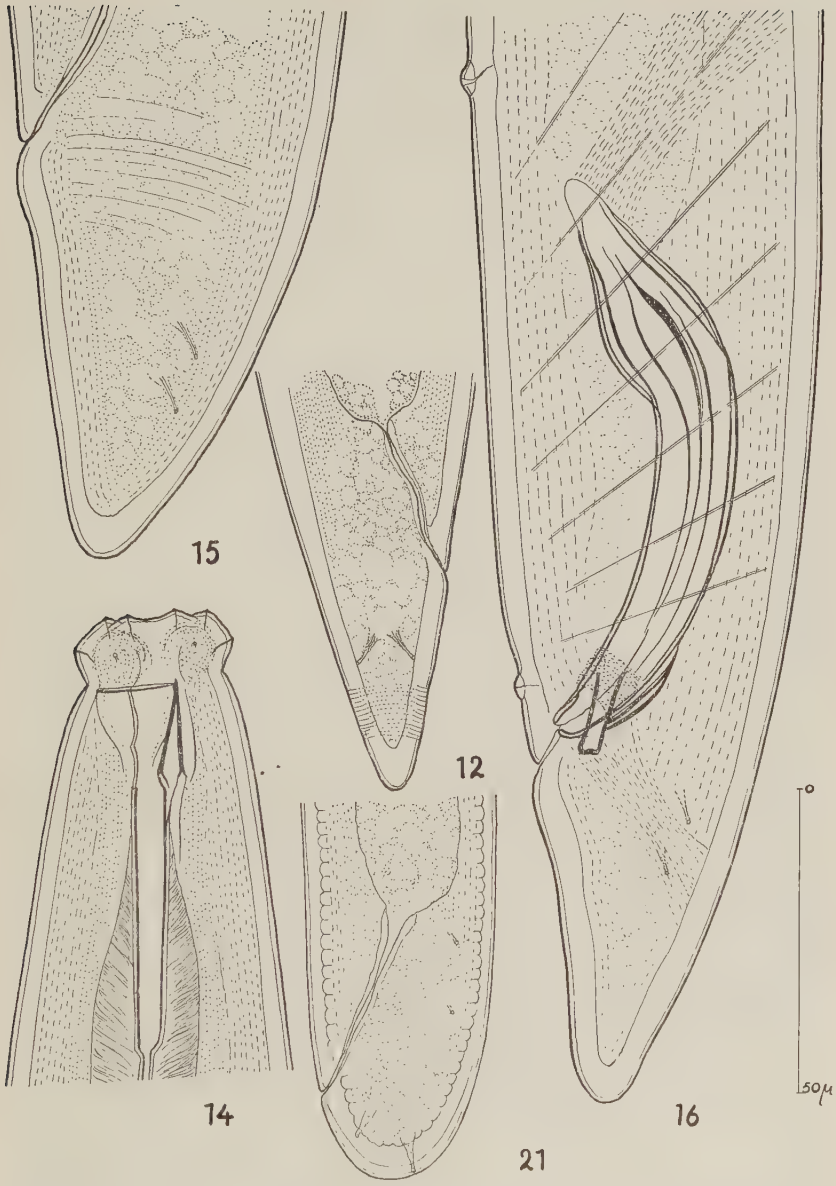
Verwandtschaft: *A. cobbi* Thorne 1937 (L = 5,0 mm, andere Anordnung der Präanalorgane). — *A. digitalis* Loos 1949 (Nur ♀ bekannt, L = 3,0-3,3 mm, Cuticula in 3 Lagen und ohne Längsstreifen und in Stachelhöhe so dick wie der Stachel, Stachelöffnung länger).

Fundort: Nur locus typicus.

Aporcelaimus paraconicaudatus n. sp. (T. III u. IV, Abb. 11-13)

♀ L = 1,4 mm a = 22 b = 3,8 c = 25 V = 53% (n = 3)
Typus: L = 1,39 mm a = 21,8 b = 3,8 c = 25,0 V = 53,4%
Locus typicus: Recife-Piedade, Station 24, Feucht sand.
♂ L = 1,0-1,3 mm a = 22-28 b = 3,5-4,0 c = 30-41 (n = 5)
Typus: L-1,30 mm a = 26,0 b = 4,0 c = 41,1
Locus typicus: Wie ♀. Coll. no. (♀) 5608; (♂) 5609.

Körper mässig schlank. Lippen sehr deutlich abgesetzt, Papillen mässig markant. Cuticula ungeschichtet, ohne Längsstreifung, mit in Punkte auflösbarer Ringelung. Lippenbreite $^1/_3$ der Körperbreite in Cardiahöhe, Seitenorgan 60% der Lippenbreite. Stachel 1 Lippenbreite lang, ca. $^1/_3$ so dick, Öffnung 60% der Länge. Ohne Führungsring, ohne Hautfalte. In Stachelhöhe Cuticularpapillen. Oesophagus nach 40% ziemlich plötzlich verbreitert, kurz vorher der Nervenring. Verdickter Teil etwa 3 mal so dick wie anfangs. Cardia 1,5 mal so lang wie breit, herzförmig. ♀ Gonaden symmetrisch (14%), mit Umschlägen bis zur $^1/_2$ Gonadenastlänge. Schwanz des ♀ konisch, am Ende stumpf, bis fast zum Ende punktiert geringelt mit zwei sehr deutlichen Papillen. Rectum etwas mehr als 1 Analbreite, Prärectum 4-5 Rectumlängen. — ♂ Gonade typisch, Beginn bei 35% der Körperlänge. Mit 14-16 einander berührenden Präanalorganen, deren Reihe wenig hinter dem proximalen Spiculumende beginnt. Gesamtausdehnung der Präanalorganreihe 90-95 μ. — Subventralpapillen fehlen. Adanales Papillenpaar vorhanden, ca. 10 μ präanal. Schrägmuskulatur deutlich. Prärectum des ♂ beginnt in Höhe des am weitesten kopfwärts gelegenen Präanalorgans. Spicula 45 μ Sehnenmass, mit lateralen Führungsstücken von $^1/_7$ Spiculumlänge (Abb. 14), distal gekerbt. Schwanz wie beim ♀, nur 2 markante Papillen vorhanden.



Tafel IV. *Aporcelaimus paraconicaudatus* n.sp.: Abb. 12 = Schwanz eines ♀. — *Nygolaimus paulbuchneri* n.sp.: Abb. 14 = Kopf eines ♀, Abb. 15 = Schwanz eines ♀, Abb. 16 = Hinterkörper eines ♂. — *Leptonchus paucipapillatus* n.sp.: Abb. 21 = Schwanz eines ♀.

Verwandtschaft: *A. conicaudatus* Altherr 1953 (Nur 1 ♀ bekannt, L = 3,25 mm, viel schlanker, kürzerer Oesophagus, andere Schwanzform).

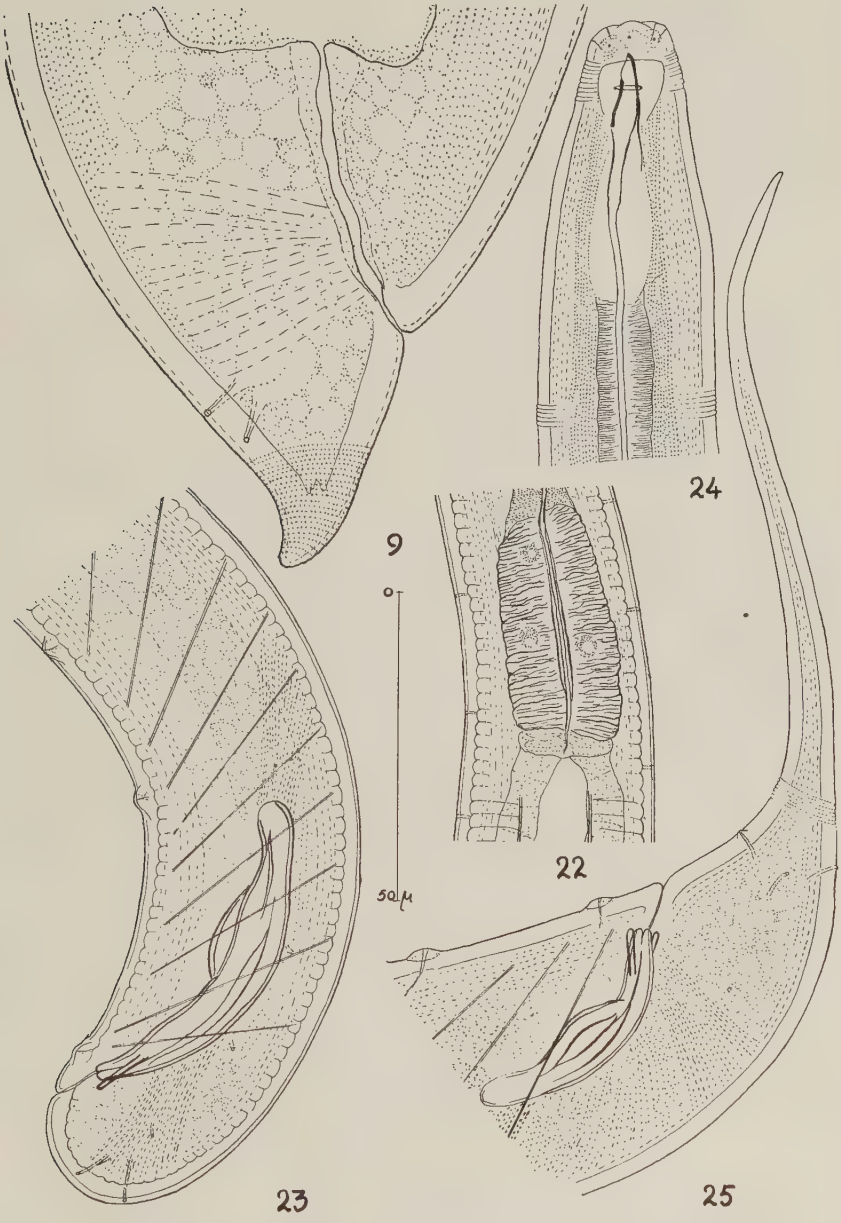
Fundorte: Ausser locus typicus noch Recife-Piedade, Feuchtsand Stationen Nr. 22 und 26. — Rio Olinda, Detritussand unter *Hibiscus*.

Nygolaimus paulbuchneri n. sp. (T. IV, Abb. 14-16)

♀ L = 3,8 mm a = 58 b = 5 c = 67-72 V = 55-59% (n = 4)
Typus: L = 3,79 mm a = 57,5 b = 5,1 c = 67,6 V = 54,8%
Locus typicus: Recife-Piedade, Station 18, Feuchtsand.
♂ L = 3,8 mm a = 61 b = 5,2 c = 58 (n = 5)
Typus: L = 3,82 mm a = 61,0 b = 5,1 c = 58,3
Locus typicus: Recife Piedade, Station 20, Feuchtsand.
Coll. no. (♀) 5610; (♂) 5611.

Körper nach vorn stark (Lippen $^1/_3$ der maximalen Körperbreite), nach hinten mässig stark (Analdurchmessers ca. $^2/_3$ max. Körperbreite) verschmälert. Cuticula glatt, ohne Längsstreifen oder Ringelung. Lippen prominent, deutlich abgesetzt, $^1/_3$ so hoch wie breit, Papillen nicht hervorstehend. Lippenbreite = 42% der Körperbreite in Cardiahöhe. Seitenorgan $^1/_2$ Lippenbreite. Stachel pfriemförmig, *Sectonema*-artig, seine ganze Länge hohl, 13-15 μ lang, = $^1/_4$ Mundhöhlenlänge bzw. $^2/_3$ der Lippenbreite (Abb. 14). Oesophagus nach 36% seiner Länge vom Kopfende an ziemlich plötzlich verbreitert, am Ende mit 3 grossen Drüsen. Nervenring vor der Verbreiterung. ♀ Gonade symmetrisch, die Äste sehr kurz (7-8%) mit Umschlägen auf 40% der Astlänge. Eier nicht beobachtet. Vulva typisch, schwach cuticularisiert. ♀ Schwanz konisch, mit deutlicher Analmuskulatur. Schwanzlänge ca. 1,3 Analdurchmesser. Rectum = 1 Analbreite, Prärectum 6-6,5 Rectumlängen. — ♂ Gonade typisch. Mit 7-8 Präanalorganen, 26 μ hinter dem proximalen Spiculaenden beginnend: Anus bis 1. Präanalorgan = 98 μ (die weiteren Abstände der einzelnen Organe: 14, 18, 13, 10, 26, 24, 13 μ), Adanales Papillenpaar vorhanden, 13 μ präanal. Sublaterale Papillen fehlen. Spicula gross, 75-78 μ Sehnenmass, Gubernakulum unscheinbar, trogförmig mit häutiger Scheide mit den distalen Spiculaenden verbunden. Laterale Führungsstücke deutlich, vorn quer angestutzt. Schwanz ähnlich dem des ♀, mit 2 Papillen.

Verwandtschaft: *N. aquaticus* Thorne 1930 (Lippen nicht abgesetzt, etwas kleiner, Stachelform anders, Stachel länger, Mund-



Tafel V. *Aporcelaimus gerlachi* n.sp.: Abb. 9 = Schwanz eines ♀. — *Leptonchus paucipapillatus* n.sp.: Abb. 22 = Cardiaregion, Abb. 23 = Hinterkörper eines ♂. —*Dorylaimoides paulbuchneri* n.sp.: Abb. 24 = Kopf eines ♀, Abb. 25 = Schwanz eines ♂.

höhle länger, Schwanz kürzer und etwas anders geformt, Präanalorganbeginn weiter kopfwärts). — *N. obtusus* Thorne 1930 (Nur ♀ bekannt, Seitenorgan kleiner, andere Schwanzform, Cardiadrüsen ungewöhnlich klein, Prärectum viel kürzer).

Fundorte: Ausser locus typicus noch: Recife-Piedade, Feuchtsand, Stationen *22*, *24* und *26*.

Leptonchus multipapillatus n. sp. (T. II, Abb. 17-19)

♀ L = 1,3 mm a = 37 b = 7,1 c = 86 V = 42% (n = 3)
Typus: L = 1,33 mm a = 36,7 b = 7,1 c = 86,0 V = 41,2%
Locus typicus: Recife-Piedade, Feuchtsand, Station *22*.
♂ (Typus) L = 1,22 mm a = 33,5 b = 5,7 c = 58,7 (n = 1)
Locus Typicus: Wie ♀. Coll. no. (♀, ♂) 5612.

Mässig schlanker Körper, nach vorn auf $^1/_3$ der maximalen Körperbreite verschmälert. Cuticula dreischichtig, die innere Lage grob geringelt. Lippen deutlicher als gattungsüblich, vom Hals etwas abgesetzt. Seitenorgan über $^2/_3$ der Lippenbreite, typisch. Stachel genustypisch, Öffnung etwa $^1/_3$ der Länge. Stachelverlängerungen von der Stachelbasis seitlich abgebogen und stärker cuticularisiert. Oesophagus bis zum Bulbus schmal und zart, schwach muskulös. Bulbus mit starker Muskulatur, *22-24* % der Oesophaguslänge lang. Cardia flach. Darmzellen nur 2 (?) im Umgang. ♀ Gonade nicht ganz symmetrisch, mit Umschlägen (nicht vermessen, da Gonade noch nicht voll ausgereift), Vulva typisch. Rectum 2 Schwanzlängen lang (Abb. 18), Prärectum nicht klar erkennbar, aber offenbar sehr weit kopfwärts beginnend. ♀ Schwanz sehr kurz, fast halbkugelig, mit 2 sehr deutlichen Papillen, davon eine fast endständig. Kopf und Hals des ♂ wie beim ♀. Mit 14 halbkugeligen Präanalorganen (ausser Adanalpapillen), die beiden ersten noch im Spiculabereich, jeweils 6-9 μ voneinander entfernt, Gesamtreihenausdehnung 110 μ. Keine Subventralpapillen. Sehr schwache Schrägmuskulatur im Präanalorganbereich. Prärectumausdehnung nicht deutlich erkennbar, aber weit vor dem am weitest kopfwärts gelegenen Präanalorgan beginnend. Spicula 30 μ Sehnenmass, mit schmalen lateralen Führungsstücken (Abb. 19). Schwanz etwas konischer als der des ♀, mit sehr undeutlichen Schwanzpapillen, von den beiden die eine fast entständig.

Verwandtschaft: Von den bisher bekannten Arten durch die grosse Zahl der Präanalorgane unterschieden.

Fundort: Wie Locus typicus.

Leptonchus paucipapillatus n. sp. (T. II, IV u. V Abb. 20-23).

♀ L = 0,8 mm a = 30-32 b = 6-7,5 c = 75-87 V = 58-60% (n = 2)
Typus: L = 0,88 mm a = 30,8 b = 7,5 c = 75,2 V = 59.8%
Locus typicus: Rio Perequé, hochgelegener Detritussand unter *Hibiscus*.
♂ (Typus) L = 0,747 mm a = 30,2 b = 6,0 c = 71,8 (n = 1)
Locus typicus: Wie ♀. Coll. no. (♀) 5613; (♂) 5614.

Körper mässig plump, Cuticula mehrschichtig, die innere Schicht grob geringelt. Seitenfeld $^1/_3$ der Körperbreite (Abb. 22). Die Lippen unterbrechen die stumpfkonische Kopfkontur nicht. Seitenorgan 1 Lippenbreite, schildförmig. Stachel genustypisch, ebenso seine Verlängerung und der Führungsring. Oesophagus, nach fast muskelloser Erweiterung um die Stachelverlängerung, auf $^4/_5$ seiner Länge schmal und sehr zart muskulös, dann ziemlich plötzlich in einen kräftigen Bulbus übergehend, der 3 deutliche Drüsenkerne besitzt und ein besonders stark kuticularisiertes Lumen aufweist (Abb. 22). Cardia eine flache Scheibe. ♀ Gonade mit einem längeren vorderen Ast (18%) und einem kurzen hinteren Ast (8%), jedoch beide mit kurzen Umschlägen ($^1/_4$ bis $^1/_3$ Astlänge). ♀ Schwanz mit am Ende zweifach geschichteter Aussencuticala, einer endständigen und einer mittelständigen Schwanzpore. Rectum 1,25 Analbreiten lang, Prärectum nur 4 Rectumlängen (!) (Abb. 21). Vorderkörper des ♂ wie beim ♀ gebaut; mit nur 2 sehr schwachen Präanalorganen (Abb. 23). Schrägmuskulatur sehr zart, Spicula anomal gross, 40 μ Sehnenmass = 2,2 Analbreiten, mit sehr schmalen lateralen Führungsstücken. Prärectumbeginn beim ♂ nicht beobachtet. Schwanz des ♂ halbkugelig gerundet, ausser den sich auch auf den Schwanz wie üblich erstreckenden Hautporen dieser Gattung, nur zwei Papillen.

Verwandtschaft: *L. granulosus* Cobb 1920 (Lippen abgesetzt, Bulbus schwächer, Prärectum weiter vorn beginnend, Spicula nur 1,5 Analbreiten, 5 Präanalorgane).

Fundort: Wie Locus typicus.

Dorylaimoides paulbuchneri n. sp. (T. V, Abb. 24-25)

♀ L = 1,2-1,4 mm a = 36-45 b = 6,1-6,3 c = 12-13 V = 40-45% (n = 7)
Typus: L = 1,23 mm a = 36,0 b = 6,1 c = 12,0 V = 40,5%

Locus typicus: Rio Perequé, hochgelegener Detritussand unter *Hibiscus*.
♂ L = 1,2 mm a = 44-50 b = 5,6-5,7 c = 12-13 (n= 2)
Typus: L = 1,23 mm a = 50,0 b = 5,6 c = 13,0
Locus typicus: Wie ♀. Coll. no. (♀) 5614a; (♂) 5615.

Körper sehr schlank, Cuticula zart, aber deutlich in Punkte auflösbar geringelt. Lippen ganz verschmolzen, die Kopfkontur kaum unterbrechend. Körperbreite in Cardiahöhe 3,5 mal grösser als Lippenbreite. Cuticularingelung in Stachelhöhe 0,6 μ Ringelbreite. Seitenorgan genustypisch, 1 Lippenbreite. Stachel asymmetrisch, 16 μ lang, an seinem Ende auf 3 μ Breite auseinanderweichend, Öffnung $^1/_6$ seiner Länge. Oesophagus nach fast muskelloser Verbreiterung auf $^2/_3$ schmal und zart muskulös, bei 45% seiner Länge vom Nervenring umfasst, dann in einen dreimal so langen wie breiten Bulbus übergehend. ♀ Gonade fast symmetrisch (G_1 = 13%, G_2 = 15%), mit Umschlägen von $^2/_3$ Astlänge. Eier lang-oval, 90: 26 μ, ♀ Schwanz im ersten $^1/_4$ konisch, dann peitschenförmig (siehe Schwanz des ♂ Abb. 25!), 5,5-6 Analbreiten lang, mit 2 deutlichen Papillenporen und einer ventralen Papille. Rectum 1 Analbreite, Prärectum 5 Rectumlängen lang. — ♂ Gonade beginnt bei 40%. Mit 6 Präanalorganen und den adanalen Papillen. Präanalorganabstände von dem dem Anus nächsten Präanalorgan an: (Anus bis 1. Organ = 33 μ): 13, 12, 14, 20 μ. Schrägmuskulatur kräftig. Spicula 30 μ Sehnenmass mit sehr schmalen lateralen Führungsstücken. Prärectumbeginn kurz vor dem am meisten kopfwärts gelegenen Präanalorgan. Schwanz wie beim ♀; ausser den 2 deutlichen Schwanzpapillen noch 2-3 sehr zarte postanale Papillen.

Verwandtschaft: *D. micoletzkyi* (De Man 1921) Thorne & Swanger 1936 (♀ Prärectum doppelt so lang, Schwanzform anders, kürzer). *D. parvus* Thorne & Swanger 1936 (kleiner, Kopf mehr konisch, andere Präanalorgane). — *D. stenodurus* Altherr 1953 (andere Stachelform, andere Stachelgrösse, grössere Spicula, Schwanz kürzer).

Fundort: Wie Locus typicus.

SUMMARY

Eight new species from the Dorylaimoidea are described from a collection made in Brazil by Dr. S. A. Gerlach, who will publish later an account of their ecology. The nematodes come from different littoral sand habitats along the Brazilian coast.

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A PRELIMINARY EXPERIMENT ON THE EFFECT OF VARIOUS CEREALS ON THE SOIL POPULATION OF CEREAL ROOT EELWORM, HETERODERA MAJOR O. SCHMIDT *)

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In 1954, forty nine microplots (JONES 1956) were constructed and filled with a mixed bulk of light, chalky soil containing cereal root eelworm (*Heterodera major* O. Schmidt) obtained from a field at Audley End, Cambridgeshire. The field was originally park land which was ploughed about 1944 and grew the following sequence of crops between 1947 and 1952: barley, kale, mustard, spring wheat, barley, oats. The oats in 1952 were a partial failure from cereal root eelworm attack. As the eelworm population in the microplots was rather low, 28.5 cysts/100 g., 8.3 eggs/g., and 29.1 eggs/cyst, the plots were all sown with oats, variety Forward, which grew satisfactorily and gave a mean final population of 39.6 cysts/100 g., 29.7 eggs/g. and 76.9 eggs./cyst. In 1955, the plots were sown with four plots each of 3 varieties of oats, 3 varieties of wheat and 6 varieties of barley (48 plots). The remaining plot was sown with Sun II oats to give 5 replicates of this particular variety. In deciding which plots the various cereals should occupy, the plots were divided into four levels of initial population and one plot of each variety was randomised within each level. In the statistical analysis of results, the levels were treated as blocks. All plots received a dressing of compound fertiliser and were sown on April 1st. Growth of the wheats and barleys was satisfactory but that of the oats rather poor, presumably because of cereal root eelworm attack. The grain and straw of the oats and barleys

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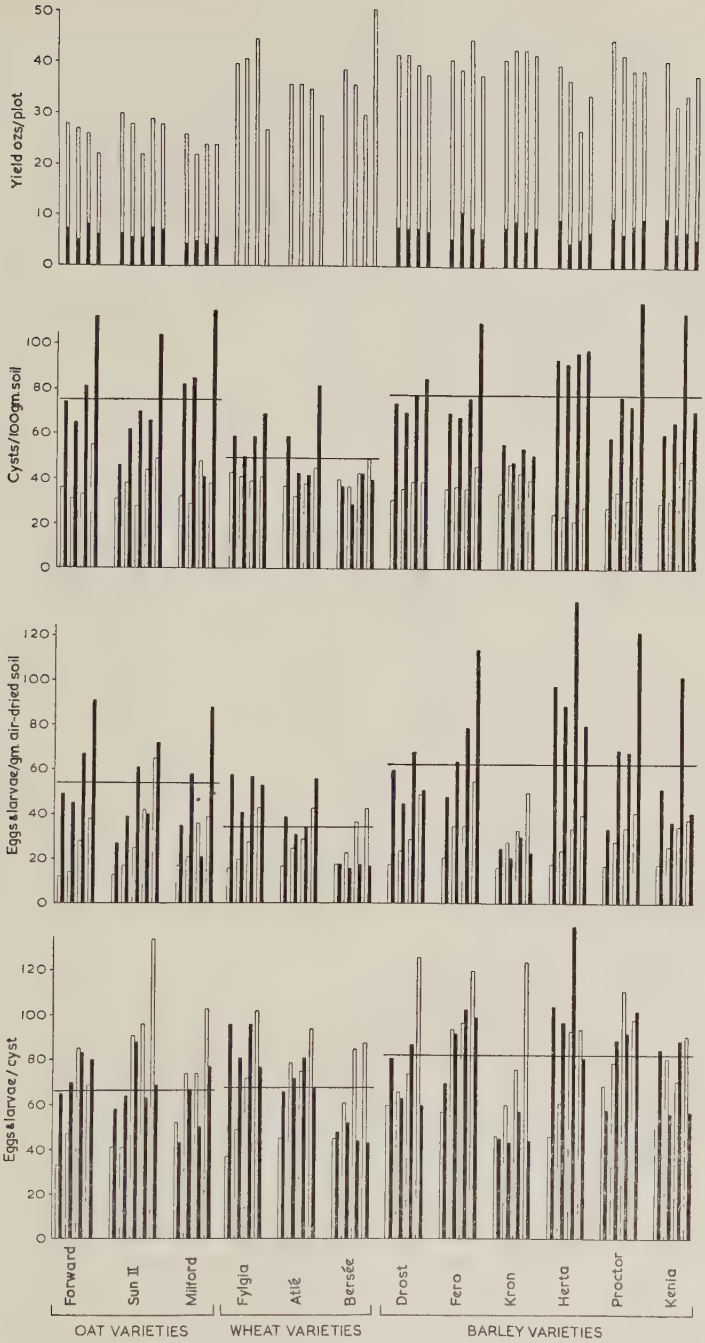

Fig. 1. Results of microplot experiment with cereal varieties. Yields: light histograms, straw; dark histograms, grain. Cysts, eggs & larvae, and eggs & larvae per cyst: light histograms, initial populations; dark histograms, final populations. Plots arranged in ascending order of initial egg population.

were harvested separately. The grain of the wheats was taken by birds despite protective netting and only straw yields are available (Fig. 1). The eelworm population of the plots before and after the cereal varieties was estimated during the winter months (November-February) on the basis of 200 g. of soil.

Results are presented in Fig. 1 and Table I. In Table I, the mean final cyst and egg counts after the various cereal varieties are given. The statistical analysis of the figures are based on the square root transformation because the Poisson error of the cyst counts varies with their size (JONES 1955). The same transformation is applied to the egg counts since these are derived from the cysts. Because of previous uniform treatment of the plots, the cyst counts give results that are almost identical with those of the egg counts and possess the advantage of greater precision, giving an S.E. % mean of 10.2% against 17.1% for eggs.

From Table I and Fig. 1, it can be seen that the oat and barley varieties produced final populations of the same order and significantly higher than those after the wheats. There was little difference between the oat varieties grown. Bersée was significantly the lowest of the wheat varieties and Kron significantly the lowest of the barley varieties. Indeed, populations after Bersée and Kron were lower than the initial populations and significantly lower than after most other cereal varieties. The highest populations followed the barley variety Herta. There were no important differences in the mean egg contents of cysts (Fig. 1), except that the values were somewhat higher for the barleys than for the oats and wheats, and that for the varieties Bersée and Kron, on which very few new cysts were formed, the mean cyst content had fallen by a small but definite margin.

In this experiment all cereals were sown on the same date and were grown under standardised conditions. When attempting to interpret the results, it must be remembered that the vegetative periods would differ in the field under farm conditions, those of the wheats especially being longer than allowed here. Also, the oat yield appeared to have been reduced by eelworm attack. Better performance of the oat varieties in less heavily infested land might have produced higher final populations. Nevertheless, the results do suggest that barleys are important in raising cereal root eelworm populations to dangerous levels: in the Audley End field the barley preceeding the damaged oats may have produced the injurious population of cereal root eelworm. Perhaps the most useful aspect of this experiment is not to be

TABLE I

The mean final cyst and egg populations of cereal root eelworm in microplots sown with various cereal varieties

		Cysts/200 g.	V	*Eggs/g.*	V
Oats	Forward	164	12.8	60.8	7.8
	Sun II	149	12.2	51.8	7.2
	Milford	156	12.5	46.0	6.8
Barleys	Herta	190	13.8	100.0	10.0
	Fero	164	12.8	74.0	8.6
	Proctor	161	12.7	68.9	8.3
	Kenia	154	12.4	54.8	7.4
	Drost	154	12.4	54.8	7.4
	Kron	102	10.1	24.0	4.9
Wheats	Fylgia	117	10.8	51.8	7.2
	Atlé	110	10.5	38.4	6.2
	Bersée	74	8.6	16.8	4.1
S.E.		—	± 0.6	—	± 0.6
Significant differences	P = 0.05	—	± 1.7	—	± 1.8
	P = 0.01	—	± 2.3	—	± 2.4
	P = 0.001	—	± 3.1	—	± 3.2
Means	Wheats	100	10.0	33.6	5.8
	Barleys	154	12.4	60.8	7.8
	Oats	165	12.5	53.3	7.3
(Mean initial population		80	—	29.7	—)

Comparisons	*Difference*	V	S.E.D	t_{33}	*Significance*
		Cysts/200 g.			
Oats v. wheat	56	2.5	± 0.50	5.0	P < 0.001
Oats v. barley	2	0.1	± 0.42	0.2	Insignificant
Wheat v. barley	54	2.4	± 0.42	5.5	P < 0.001
		Eggs/g.			
Oats v. wheat	16.7	1.5	± 0.50	3.0	P < 0.01
Oats v. barley	7.5	0.5	± 0.43	1.2	Insignificant
Wheat v. barley	27.2	2.0	± 4.43	4.5	P < 0.001

S.E. per plot % mean: cysts 10.2%; eggs 17.1%

found in the face value of the results but in the demonstration that the microplot technique can be successfully used to measure differences in host efficiency between cereal varieties. These differences appear to be less clear cut and therefore more difficult to assess than with the hosts of the beet eelworm (*Heterodera schachtii* Schm.) or the pea root eelworm (*Heterodera göttingiana* Liebscher) (JONES 1956, JONES and MORIARTY 1956, WINSLOW 1955).

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SUMMARY

In an experiment with microplots, oats and barley caused greater increases in cereal root eelworm populations than did wheat. The highest population followed barley, variety Herta. A slight decline in population followed barley, variety Kron, and wheat variety Bersée, both of which gave final populations significantly lower than most of the other cereal varieties tested. Because of the previous uniform treatment of the plots, population changes were adequately measured by cyst counts which gave greater precision than the egg counts.

ZUSAMMENFASSUNG

In einem Mikroparzellenversuch brachten Hafer und Gerste eine stärkere Vermehrung der Hafernematodenpopulationen als Weizen. Die höchste Population folgte nach Gerste, Sorte Herta. Eine leichtes Absinken der Population trat nach Gerste, Sorte Kron und nach Weizen, Sorte Bersée ein. In beiden Fällen waren die Schlusspopulationen beträchtlich niedriger als bei den meisten anderen geprüften Sorten.

Wegen der vorhergehenden einheitlichen Behandlung der Parzellen wurden die Populationsänderungen durch Zystenzählungen ermittelt, die eine grössere Genauigkeit als Eizählungen ergaben.

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THE PRODUCTION OF STERILE VIABLE LARVAE OF THE POTATO ROOT EELWORM, *HETERODERA ROSTOCHIENSIS* *)

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The experiments herein described were carried out as a preliminary to setting up cultures of *Heterodera rostochiensis* Woll. in excised tomato roots growing in tissue culture. This demands rigid asepsis and anything introduced into a culture must therefore be sterile. In this work it was necessary to sterilise larvae of *H. rostochiensis* without destroying their viability.

A number of workers have attempted to free nematodes from associated micro-organisms. ZIMMERMANN (1921) working on vinegar eelworm, *Turbatrix aceti* (Muller, 1783) freed the worms by prolonged washing in sterile water. BRIGGS (1946) treated eggs of *Rhabditis elegans* (Maupas 1900) for 15 minutes in 1: 500 chlorox solution, washed them with sterile water and exposed them to ultraviolet light (200 μ watts/cm^2) for 30-35 seconds. DOUGHERTY & CALHOUN (1948) attempted to sterilise *Rhabditis pellio* (Schneider 1866) and *R. elegans* using antibiotics: the worms were transferred by capillary pipette to a broth containing 5,000 units of penicillin and 5,000 units of streptomycin per ml. After 24 hours the larvae were rinsed with sterile water and transferred to a 1: 1,000 aqueous solution of merthiolate for 1 hour, the viable larvae were then rinsed once or twice in sterile water containing 1,000 units of penicillin per ml. NICHOLAS & ENTEGART (1955) worked on *Caenorhabditis briggsae*, (Dougherty & Nigon, 1949) (= *Rhabditis briggsae*), *Rhabditis maupasi* (Seurat 1919) and on *R. axei* (Cobbold, 1884) and successfully evolved two methods for sterilization: the first, known as the merthiolate method, involved the transfer of gravid females to an 0.5 % agar containing 0.1 % merthiolate for a period of 1½ hours with subsequent transfer to an antibiotic, 0.25 % agar containing 1,000 units of penicillin and streptomycin and 250 units of chloromycetin

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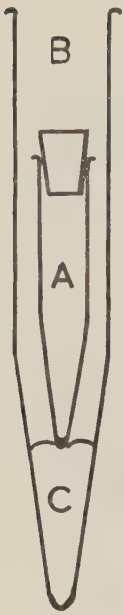

Fig. 1. Showing arrangements for aseptic centrifuging of eggs aud larvae.

per ml. for up to 48 hours; the young worms being subsequently transferred to sterile saline. The second method utilised hydrogen peroxide; worms were treated with 10 volume hydrogen peroxide for 10 minutes, washed with sterile water and then transferred to an antibiotic solution containing 1,000 units penicillin and 250 units streptomycin per ml. for 24-48 hours according to the species of worm; subsequent washing in sterile water eliminated the antibiotic.

All these methods suffered from one disadvantage, they were rather complicated and involved a lot of handling of the experimental material. The author's previous unpublished experiences with the eggs and larvae of *Ascaris lumbricoides* (Linnaeus 1758) indicated that asepsis could be accomplished with less complicated procedures, which did not involve the use of antibiotics. In the case of *Ascaris* the only procedure necessary was the treatment of the mature eggs of the worm with a $^1/_{20}$ solution of "Milton" for 10-15minutes, followed by washing in sterile water. The larvae could then be liberated by gentle squashing between sterile slides; the larvae thus obtained were sterile and appeared to be in every respect healthy and viable and were subsequently used for culture experiments.

TECHNIQUE

Eggs and larvae of *H. rostochiensis* were obtained from a number of stocks of cysts normally kept in the laboratory. Batches of up to 1,000 cysts were soaked in water at 25° C. for 7-14 days and then transferred to potato root diffusate of L.A. value 3.0-3.5. The larvae were removed daily by means of pipette for experiment, none of the material being more than 24 hours old. Eggs were obtained by a different method. Batches of up to 1,000 cysts, after preliminary soaking, were dissected either by the Hagedorn needle technique (Reid, 1952) or by Reid's rolling slide technique (1955). The eggs and larvae thus liberated were agitated by means of a Horlick's mixer and then sieved through bolting silk to eliminate particles of cyst wall. The larvae were removed from the suspension by a differential sedimentation technique to be described later in another paper. The resulting suspension contained not more than 5-10 % larvae.

The transfer of eggs or larvae from one solution to another was accomplished by centrifuging, small centrifuge tubes being used for this purpose (Fig. 1). Each tube (A) was made from 10 mm. glass tubing drawn out to a coarse thick walled capillary, which was cut off and sealed; by holding the sealed end in the flame the lower part of the tube thickened and the resulting increased strength enabled the tube to withstand the stresses of centrifuging. The top of the tube was reamed to form a rim to faciliate the fitting and removal of corks. For centrifuging, these small tubes were supported inside an ordinary 15 ml. centrifuge tube (B) on a cotton wool pad (C). A hand centrifuge was found to be more convenient than an electric centrifuge and centrifuging was carried out for about 1 minute at approximately 1,000 r.p.m. The addition and removal of fluid was accomplished by sterilized pipettes.

When eggs or larvae were treated with different substances for short times (up to 20-30 minutes), this was done in the corked sterile centrifuge tubes which were continuously agitated by hand over the treatment period. When treatment was extended for several hours, eggs and larvae were transferred to sterile watchglasses enclosed in sterile Petri dishes for the appropriate times and returned to the centrifuge tubes for washing.

All glassware was sterilized at 110° C for 30 minutes. Root diffusate was sterilized by filtration, other fluids, water etc. were sterilized by autoclaving. After treatment, eggs or larvae were inoculated into sterile nutrient broth and incubated at 24° C. It was found necessary to continue incubation for four weeks as some of the sterilants used, especially in the case of egg sterilization delayed the growth of bacteria till the third and fourth week.

Viability of larvae was investigated by inoculation tests on young tomato seedlings. The latter were grown in 3-in. pots of sand. Treated larvae were introduced into the sand around the roots when the seedlings were about 7-10 days old. Three such pots were used in any one treatment and a similar set of inoculations with untreated larvae were used as controls. Seven days after inoculation, the roots were removed, washed and stained in acid fuchsin in lactophenol; after differentiating in clear lactophenol the number of larvae present in the roots was counted and comparisons made between treated and untreated larvae to obtain an estimate of viability loss in sterilizing.

The viability tests on eggs were a little more complicated. The eggs after sterilization were transferred to potato root diffusate and allowed

to hatch for one week. The number of eggs and larvae present at the beginning and end of this period was estimated by dilution and in this way an estimate was made of the number of eggs which hatched. Comparison of the hatching of untreated and treated eggs gave a first approximation to loss of viability on treatment. The larvae emerging from the eggs were collected and tested for viability by inoculation into pots of tomato plants as before.

PROCEDURE

Sterilization of larvae

Preliminary tests on larvae were made with a 1: 20 dilution of "Milton" [1]). Treatment of larvae with this substance for 10 minutes resulted in complete asepsis; the larvae remained active and appeared in all respects to be normal and healthy. When tested for invasive power in either agar or sand culture, vast numbers congregated around the growing root tips but none appeared to be able to penetrate the root surface. When kept in shallow layers of water, they retained their activity for 1 to 2 weeks and in this respect compared very favourably with untreated larvae. When introduced into the neighbourhood of young roots however, they lost their activity within a period of 24-48 hours. More dilute solutions of "Milton" were tested and gave similar results — treatment with 1: 120 "Milton" for 10 minutes resulted in asepsis being achieved in about 50 % of cases but with complete loss of the power of invasion.

Further tests were made using hydrogen peroxide with disappointing results. Twenty minutes treatment with a 20 volume solution failed to achieve asepsis but resulted in loss of viability. The use of more dilute solutions for longer times gave similarly disappointing results.

A number of other compounds were tested. Ethoxy ethyl mercury chloride at concentrations ranging from 5-20 p.p.m. for up to 24 hours failed to achieve asepsis and killed the larvae. 8 hydroxy quinoline potassium sulphate tested at 1 %-0.25 % for up to 72 hours was also unsuccessful in achieving asepsis. Dehydro acetic acid in saturated solution for up to four hours failed to sterilise.

Treatment of eggs

The tests with larvae having proved disappointing, attention was

1) This mixture contains a 1% solution of sodium hypochlorite and 16.5% sodium chloride. It is manufactured by the "Milton Antiseptic Co. Ltd." of London.

paid to the sterilization of eggs and again "Milton" was tested. Treatment with 1: 20 Milton for 10 minutes failed to achieve asepsis; the majority of the larvae emerged from the eggs and were directly exposed to the action of the hypochlorite so that even if they had been sterile, it is unlikely that they would have retained their invasive power. Tests with weaker solutions of "Milton" for longer times were equally unsuccessful in achieving asepsis and the tendency for the larvae to emerge from the eggs persisted even at 1/80.

Examination of the eggs showed that they were covered with a layer of mucilaginous material which might have prevented the antiseptic reaching the egg shell, so attempts were made to disperse it prior to sterilization. Trypsin was used for this purpose, for periods ranging from 1 to 16 hours; the mucilaginous material seemed to disappear after a matter of 1-2 hours, but subsequent treatment of the eggs with "Milton" did not result in asepsis.

A number of other sterilizing agents were tested without success; they included ethoxy ethyl mercury chloride, 8 hydroxy quinoline potassium sulphate and dehydro acetic acid, all of which were tested at the same concentrations as for larval sterilization, with and without pretreatment with trypsin.

Asepsis was finally achieved using hydrogen peroxide. It was found that 5 hours treatment with 20 volumes of hydrogen peroxide or 24 hours treatment with a 5 volume solution resulted in asepsis. After several tests it was found most convenient to treat eggs with a 20 volume solution for 8 hours and the following technique was developed. Eggs were recovered from cysts and transferred to small corked sterile centrifuge tubes and spun down. The liquid was removed with a sterile pipette and replaced by hydrogen peroxide; after agitation the eggs were again spun down, the peroxide replaced by fresh peroxide and the eggs again agitated. The egg suspension was then transferred to sterile watchglasses enclosed in sterile Petri dishes and allowed to stand for 8 hours; it was then transferred by sterile pipette to sterile centrifuge tubes and washed twice with sterile water. The eggs were inoculated into sterile broth to test for asepsis; only in about 10-15 % of cases was there evidence of contamination after 4 weeks incubation.

Egg hatch was tested by treatment with potato root diffusate. No loss in hatchability was detected as a result of 8 hours treatment with 20 volume peroxide but there was some evidence that 5 hours treatment actually stimulated larval emergence. 24 hours treatment

was harmful, larval emergence fell by about 50 %. The hatched larvae appeared normal and healthy and were active. Inoculation tests using tomato seedlings grown in sand disclosed no appreciable loss of invasive power as compared with untreated larvae.

SUMMARY

Experiments were performed on the sterilization of eggs and larvae of *Heterodera rostochiensis*. Larvae could be sterilized using a 1/20 dilution of "Milton" but this resulted in loss of the power of invading roots. Treatment of eggs with sterilizing agents was more difficult but 8 hours treatment with hydrogen peroxide resulted in complete asepsis with no loss of hatchability or invasive power.

ZUSAMMENFASSUNG

Versuche zur Sterilisierung von Eiern und Larven des Kartoffelnematoden (*Heterodera rostochiensis* Woll.) wurden durchgeführt. Larven konnten unter Verwendung von 0,05% "Milton" sterilisiert werden, aber hierdurch verloren sie die Fähigkeit, in die Wurzeln einzudringen. Die Eibehandlung mit sterilisierenden Stoffen war schwieriger, aber eine 8 stündige Behandlung mit Wasserstoffsuperoxyd ergab völlige Asepsis ohne Verlust der Schlüpf- oder der Eindringungsfähigkeit.

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(*Nematologica*, Vol. I, 1956, no. 4)

THE AXENIC CULTURE OF TURBATRIX ACETI (THE VINEGAR EELWORM) [1])

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An early contribution to the study of nematode nutrition was made by ZIMMERMANN (1921) who found that although the bacteria present in vinegar and other fermenting media formed an essential part of the diet of the vinegar eelworm, *Turbatrix aceti* (Müller 1873) Peters he could culture the worms, with continued reproduction, in a bacteria-free (axenic) solution of peptone, glucose, lecithin, sodium chloride and a yeast extract. Recently ELLS and READ (1952) failed to culture *T. aceti* in this medium but reported that they could do so when an ether extract of rat liver was substituted for the lecithin.

In view of recent work on the nutrition of other nematodes (DOUGHERTY, 1951; 1953; DOUGHERTY and KEITH, 1953; DOUGHERTY, RAPHAEL and ALTON, 1950; GLASER, 1940; GLASER, MCCOY and GIRTH, 1942; STOLL, 1953; WEINSTEIN, 1953) where more complex media have been found necessary, it seemed worth while to repeat ZIMMERMANN's and ELLS and READ's experiments and also to try some of the media which have been successfully used by DOUGHERTY (1951; 1953) for the axenic culture of *Caenorhabditis briggsae* (Dougherty and Nigon 1949) Dougherty 1953.

In my experiments with ZIMMERMANN's and ELLS and READ's media, the worms survived for several months but reproduction was not sustained. It must be borne in mind, however, that their culture media cannot be copied exactly because of the chemically undefined nature of some of the constituents, but it is also noteworthy that in neither of the previous studies was serial sub-culturing reported. Experiments with two of Dougherty's media were carried out in the following way.

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1) Received for publication: May 7, 1956.
2) This work was carried out while the author was a holder of a Beit Memorial Fellowship.

THE BACTERIOLOGICAL STERILIZATION OF THE WORMS

Bacteriologically sterile worms to initiate the cultures were obtained by a modification of Zimmermann's method which makes use of hydrogen peroxide as a sterilizing agent. In my experiments, antibiotics have also been used.

Worms taken from a culture in vinegar were washed in hydrogen peroxide (10 vols. per cent) for ten minutes, and then with three changes of sterile tap water, daily for ten days. During the intervals the worms were kept in sterile Ringer's solution rendered bacteriostatic with penicillin and streptomycin. It was found convenient to keep the worms thoughout the sterilizing procedure in a covered Seitz filter over a filter pad of the clarifying grade (suggested by LEIPER, personal communication). In this way, successive solutions could be drawn off aseptically while the worms were retained in the filter by the pad. Mortality was heavy, but by starting with large numbers, a plentiful supply of bacteriologically sterile worms was obtained.

Bacteriological sterility was checked by inoculating samples of the worms into nutrient agar, glucose peptone agar and Brewer's thioglycollate broth. These were then kept for fourteen days at room temperature, followed by seven days at 37° C. The same methods were used when testing the worm cultures for the presence of contaminating organisms. Various other bacteriological media were tried, e.g. Sabouraud's medium, and slopes of Dougherty's culture media (see below) solidified with agar. These media did not show the presence of contaminants missed by the standard test media. Only a small proportion of the worm cultures were shown to be contaminated, usually with fungi. All the contaminated cultures were discarded.

PREPARATION OF THE CULTURE MEDIA

Both the culture media used contained an autoclaved liver homogenate, but one was supplemented with an unheated liver extract, and the other with an unheated chick embryo extract. The autoclaved liver homogenate was prepared from 250 gms. of bovine liver and 250 mls. of M/15 Sorenson's phosphate buffer (pH 7) in a Waring Blender. The homogenate was autoclaved and the resulting clear amber liquid poured off from the protein clot, filtered, distributed in 2 ml. quantities in small test tubes, plugged with cotton wool, and re-autoclaved.

The unheated liver extract was prepared from a similar liver homogenate which was then centrifuged at 20,000 G for two hours in a

chilled centrifuge. The supernatant was fractionated with ammonium sulphate, and the fraction precipitating at between 40% and 60% saturation was redissolved in the M/15 phosphate buffer and sterilized by Seitz filtration. The extract was used to supplement the autoclaved homogenate by adding 0.5 ml. to each 2 ml. of the homogenate. To prevent deterioration in storage, the extract can be lyophilised, either before of after Seitz filtration.

The chick embryo extract was prepared aseptically from ten-day-old embryos. These were homogenized in an approximately equal quantity of sterile distilled water, then, after centrifuging at moderate speed, the supernatant was distributed in ampoules, which were sealed after lyophilisation. The extract was reconstituted with sterile distilled water and added at the rate of 0.5 ml. to every 2 ml. tube of the autoclaved homogenate immediately after the tube had been inoculated with worms.

RESULTS

Thriving cultures were obtained by inoculating bacteriologically sterile worms into either type of medium. These have been maintained for more than a year by serial sub-culturing, when 0.1 ml. of the culture was transferred to a fresh culture tube aseptically. The concentration of the worms in the old culture was estimated by taking a further 0.5 ml. and, after suitable dilution, counting the worms in an eelworm counting slide. All cultures were kept at room temperature.

Cultures containing tens of thousands of worms were eventually obtained, but the rate of reproduction and the final numbers attained were much higher in the cultures supplemented by chick-embryo extract. These required sub-culturing at approximately monthly intervals while the liver supplemented cultures were only sub-cultured at two- or three-monthly intervals. In unsupplemented cultures, reproduction is not sustained.

A more quantitative assesment of the culture medium has not been made. Counts reveal that, although, as noted above, the cultures supplemented by the chick embryo extract produced more worms than those supplemented with liver extract, there was considerable variation among the cultures of each type. The media themselves are unstandardised and unstable and it has been decided to try to develop a better defined culture medium before attempting to make a more detailed study of growth and reproduction in such axenic cultures.

ACKNOWLEDGEMENTS

I should like to thank Dr. E. C. Dougherty, of the University of California; Dr. J. W. G. Leiper, of Boots Chemists, Veterinary Research Division; Dr. M. G. McEntegart, of the University of Aberdeen; and Evans Biological Institute, Runcorn, in particular Mr. G. Shaw; for their assistance.

SUMMARY

Culture media in which *Turbatrix aceti* (Müller 1873) Peters 1927 can be cultivated under bacteriologically sterile conditions have been described in the past. The author has unsuccessfully attempted to confirm these findings, but has succeeded in culturing *T. aceti* with continued growth and reproduction for more than a year in sterile conditions by serial sub-culture. Two types of media, similar to those previously used for another free-living nematode, *Caenorhabditis briggsae* (Dougherty & Nigon 1949) Dougherty 1953, were successfully employed.

The preparation and use of these media are described.

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(*Nematologica*, Vol. I, 1956, no. 4)

AN EXPERIMENT ON THE EFFECT OF A PREDACIOUS FUNGUS UPON THE SOIL POPULATION OF POTATO ROOT EELWORM, HETERODERA ROSTOCHIENSIS WOLL. *)

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In 1955 an attempt was made to assess the effects of a predacious fungus (DUDDINGTON 1955) together with organic matter upon the soil population of potato root eelworm. The experiment was conducted in microplots (JONES 1956) of uniform initial population level in the form of a 7 × 7 Latin square, the relevant data being set out in Table I below:

TABLE I

Data relating to the microplot experiment on predacious fungi

Treatments:

1.	None	
2.	Fungus cultured in sterile leaf mould	2 lb./plot
3.	Sterile leaf mould	2 lb./plot
4.	Compost 2 lb./plot, fungus mycelium	¼ lb./plot
5.	Compost	2 lb./plot
6.	Chopped cabbage 2 lb./plot, fungus mycelium	¼ lb./plot
7.	Chopped cabbage	2 lb./plot

Lay-out: 7 × 7 Latin square
Fungus: Dactylaria thaumasia Drechsler
Initial Eelworm Population: 80 cysts/100 g.
10 eggs/g.
Plot Size: 1/6,000 acre (1 lb./plot = 2.7 tons/acre)
Potato variety: Majestic
Sowing date: 11th May
Harvesting date: 15th Sept.
Fertilizer: National Growmore 10 cwt/acre, all plots
Soil: Light sandy loam
Predacious Fungi
The following species occurred naturally in the soil:
Arthrobotrys oligospora Fres.
A. conoides Drechsler

*) Received for publication: March 2, 1956.

The soil for the microplots was obtained from Sandy, Bedfordshire and mechanically mixed before being placed in the plots (Jones 1956). The fungus mycelium for treatments 4 and 6 was produced in bulk in deep culture. Both fungus and organic matter were spread evenly over the plots and forked into the surface soil shortly before planting.

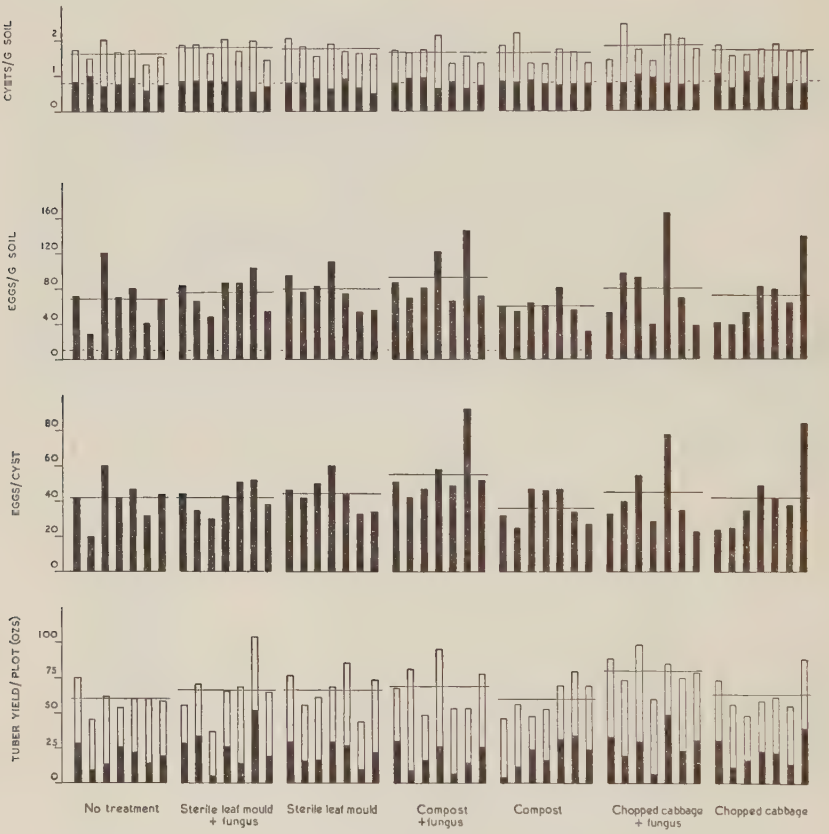

Fig. 1. Results of the microplot experiment on predacious fungus and organic matter against potato root eelworm. Cysts: dark histograms, initial populations; light histograms, final populations; dotted line, mean initial cyst population; continuous lines, mean final cyst populations. Eggs: dotted line, mean initial population; continuous lines, mean final populations. Eggs per cyst: continuous lines, mean final egg contents of cysts. Tuber yields: dark histograms, ware; light histograms, total yield (ware + chats); continuous lines, mean total yields from the various treatments. The plots are in random order.

The quantities of fungus and organic matter applied were far greater than would be possible on a field scale. The initial eelworm population

of the plots (10 eggs/g. soil) was lower than intended and appeared to have been 'diluted' during the process of collecting and mixing compared with the level obtained from superficial samples taken at the site where it was obtained. The weather was cool and moist during May and June, the period during which the plants would be subject to maximum invasion by eelworm larvae, followed by hot dry weather in July and August. No symptoms of potato sickness developed.

The results of the experiment are shown in Fig. 1. Addition of the three forms of organic matter, with and without fungus, produced no significant effects upon the cyst and egg populations of potato root eelworm or upon the potato yields.

The authors wish to thank Boots Pure Drug Co., Ltd., for culturing the fungus in bulk, Mr. D. G. Twomey for assistance in applying the treatments and Miss J. Wood for assistance with the cyst and egg counts. The experiment was financed by a grant from the Agricultural Research Council.

SUMMARY

A microplot experiment was made to test the effect of a predacious fungus (*Dactylaria thaumasia* Drechsler) and three types of organic matter (leaf mould, compost and chopped cabbage) upon potato root eelworm (*Heterodera rostochiensis* Woll.). The initial population of the eelworm was low and the treatments were without effect upon the yield of potatoes or upon the final cyst and egg populations.

ZUSAMMENFASSUNG

In einem Mikroparzellenversuch wurde die Wirkung eines räuberischen Pilzes (*Dactylaria thaumasia* Drechsler) und dreier verschiedener Arten organischer Substanz (Blatterde, Kompost und kleingeschnittener Kohl) auf *Heterodera rostochiensis* Woll. geprüft. Die Ausgangspopulation der Nematoden war klein, und die Behandlungen blieben ohne Einfluss auf die Höhe der Kartoffelernte und auf die Anzahl der Zysten und Eier bei Versuchsende.

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(*Nematologica*, Vol. I, 1956, no. 4)

THE EFFECT OF PREDACIOUS FUNGUS AND ORGANIC MATTER UPON THE SOIL POPULATION OF BEET EELWORM, HETERODERA SCHACHTII SCHM. [1])

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In 1955, a microplot experiment (JONES 1956) was undertaken to assess the effect of predacious fungi (DUDDINGTON 1955), with and without organic matter, upon the beet eelworm population of heavily infested soil. Details of the experiments are set out in Table I. The microplot soil, which was obtained originally from Chatteris,

TABLE I

Details of the microplot experiment with predacious fungi.

Treatments

1. None
2. Organic matter only (bran)
3. Fungal mycelium only
4. Organic matter and fungal mycelium
5. Organic matter and two doses of fungal mycelium
6. Organic matter and two fungi with different growing rates

Fungus Treatments 3-6, 1 lb./plot of the mycelium of *Dactylaria thaumasia*.
Treatments 5, second dose of *D. thaumasia* applied in mid-August.
Treatment 6, 10 ml. of a culture of *Dactylella doedycoides*/plot in addition to *D. thaumasia*.
Organic matter: Treatments 2, 4-6, bran at 3 lb./plot.
Lay-out: 6 × 8 randomised block.
Initial eelworm population: 346 cyst/100g.
117 eggs/g.
Plot size: 1/6,000 acre (1 lb./plot = 2.7 tons/acre)
Sugar Beet: Sharpe's British Klein
Sowing date: 1 April
Harvesting date: 7 October
Fertiliser: 5 cwt Fisions Market Garden No. 4.
Soil: Fen skirt, peaty silty loam.

1) Received for publication March, 2, 1956.

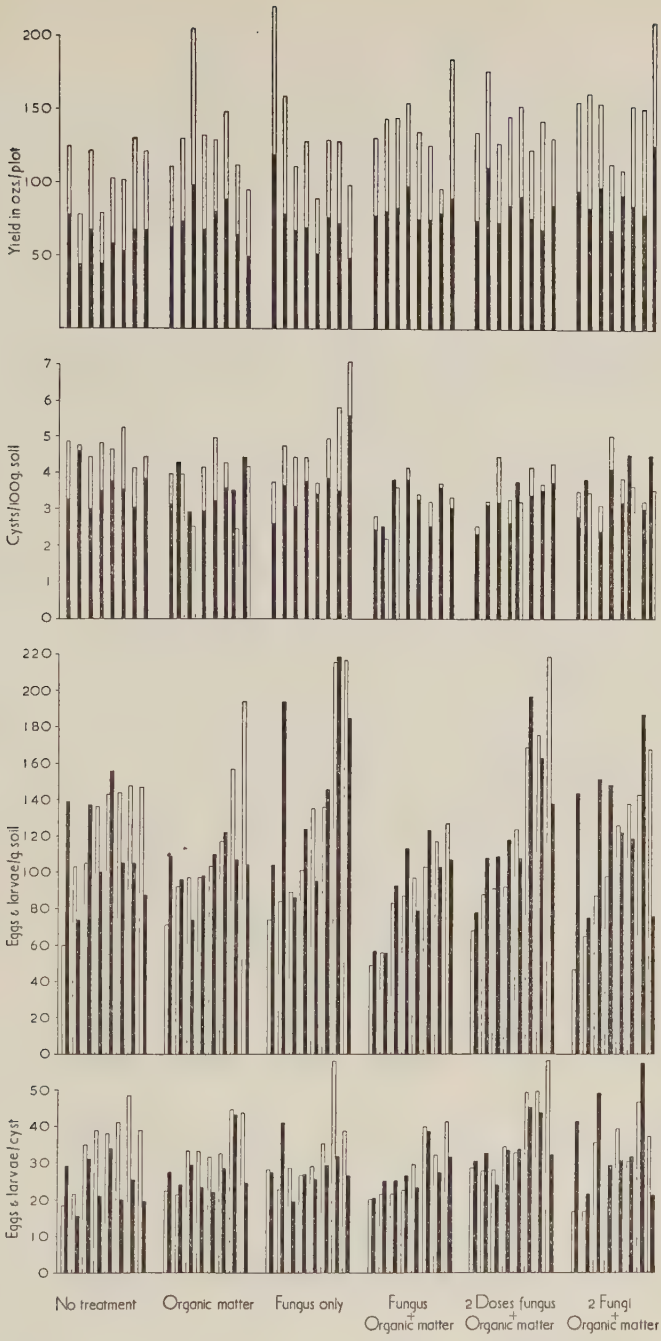

Fig. 1. Results of the microplot experiment with fungus and organic matter. Yields: dark histograms, roots; light histograms, tops. Cysts: dark histograms, initial population; light histograms, final population. Eggs: light histograms, initial population; dark histograms, final population. Eggs per cyst: light histograms, initial population; dark histograms, final population. The plots are not in random order but in ascending order of initial egg population for each treatment, so as to show any relationship that might exist between yield and initial population.

had grown beet in the two preceeding years and had received no organic matter for more than five years. It contained a natural infection of the predacious fungus *Arthrobotrys oligospora* Fres. The mycelium of *Dactylaria thaumasia* Drechsler for treatments 3-6 was produced in bulk in deep culture. *Dactylella doedycoides* Drechsler was cultured in ¾ inch test tubes each containing 10 ml. of maizemeal broth.

Population counts and yield data from the trial are given in Fig. 1 and Tables II and III. The application of organic matter at very high

TABLE II

Summary of results, means of cyst, egg, and yield data.

	Treatments							
	None	Organic matter	Fungus	Fungus + Organic matter	2 doses of fungus + Organic matter	2 fungi + Organic matter	SE*	Significance
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	
				Cysts/100g.				
Initial population	358	351	372	316	335	345	± 25.1	Insignificant
Less 20% decay **	286	281	298	253	268	276		
Final population	467	383	488	333	363	370	± 25.7	Significant $P < 0.001$
Estimated increase	181	102	190	80	95	94		
				Eggs/g.				
Initial population	124	116	133	91	129	111	± 15.0	Insignificant
Final population	114	103	144	94	128	128	± 12.7	Insignificant
				Yield in ozs./plot				
Roots	60	75	75	84	84	92	± 5.0	Significant
%	100	124	122	139	140	150	± 8.3	$P < 0.01$
Root + tops	108	134	134	141	143	152	± 8.0	Significant
%	100	124	124	131	133	141	± 7.4	$P < 0.01$

* Three times the SE may be taken as the least significant difference between treatment means.

** The estimated mean annual rate of cyst decay for Chatteris soil (Jones 1956).

TABLE III

Comparisons of the effects of fungus mycelium and organic matter upon the final cyst population. Cysts/100g.

No fungus	*Fungus*	*Difference*	*SE of difference*	*t*	*n*	*Significance*
425	389	— 36	± 22.3	1.6	35	Insignificant
No organic matter	*Organic matter*					
477	362	— 115	± 22.3	5.3	35	Significant $P < 0.001$

rates significantly affected the final cyst populations of the plots but not the final egg populations. Differences in the final cyst populations appeared to be due to the organic matter rather than the fungus (Table III). The treatments applied also affected yield. Organic matter alone (col. 2) and fungus alone (col. 3) both increased yields equally (22-24%), while the two together (col. 4) gave increases less than the sum of the individual effects (31-39%); there was no evidence of a positive interaction between fungus and organic matter. Organic matter and two doses of fungus (col. 5), or two fungi with different growth rates (col. 6)), gave no significant increase over one dose of fungus, although the two fungi together gave the highest yields (41-50% increase over controls). The heavy initial eelworm population of all plots gave rise to the usual symptoms of eelworm attack which were most prominent in July. The yield of the control plots was equivalent to 10 tons/acre, about half that normally expected from similar microplots with low eelworm populations.

In this experiment, there was no means of deciding whether the fungal mycelium, added in such excessive quantities to the soil, was actively destroying eelworm larvae during the invasive stage, or whether it broke down and behaved as a second form of organic matter. As organic matter, however, it would be much less in quantity than the bran, because the mycelium contains more than 80% of water. The decrease in cyst numbers, apparently caused by the application of bran, may have arisen in a variety of ways other than by activation of predacious fungi. For example, the bran, by increasing the microflora and fauna of the soil with resultant increase in competition for oxygen, may have reduced the rate of hatch (Wallace 1956) and the activity of the beet eelworm larvae. Further work is required to elucidate these points.

The authors wish to thank Mr. D. G. Twomey for assistance in applying treatments, to Mr P. Halfpenny for assistance with the soil population estimates and to Boots Pure Drug Co., Ltd., for supplying the fungus in bulk. The experiment was financed by grants from the Committee for Sugar Beet Research and the Agricultural Research Council.

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SUMMARY

In microplots containing soil very heavily infested with beet eelworm (*Heterodera schachtii* Schm.), an experiment was undertaken to test the effect of heavy doses of fungal mycelium and organic matter upon the yield of beet and the final eelworm population. Both fungus (*Dactylaria thaumasia* Drechsler) and organic matter (bran) increased the yield of beet. Fungus had no significant effect upon the final cyst population but organic matter caused a significant depression. Neither treatment affected the final egg population. It is not known whether the organic matter lowered the cyst population by enhancing the effect of predacious fungi in the soil or in some other way, e.g. by increasing the micro-fauna and flora, thus leading to increased competion for oxygen, which might reduce the hatch and activity beet eelworm larvae.

ZUSAMMENFASSUNG

Stark mit *Heterodera schachtii* Schm. verseuchter Boden wurde im Mikroparzellenversuch mit hohen Dosen Pilzmycel (*Dactylaria thaumasia* Drechsler) und organischem Material (Kleie) behandelt. Beide Faktoren erhöhten die Rübenernte. Der Pilz hatte keinen merklichen Einfluss auf die abschliessende Zystenzahl, aber die Kleie verursachte eine deutliche Minderung. Die Anzahl der Eier wurde in keinem Falle beeinflusst. Es ist noch nicht bekannt, ob das organische Material die Zystenpopulation durch eine Förderung der räuberischen Pilze im Boden verminderte oder auf einem anderen Wege. So könnte z.B. durch das Anwachsen der Mikrofauna und -flora ein Sauerstoffmangel eintreten, der das Schlüpfen und die Vitalität der Nematodenlarven beeinträchtigt.

(*Nematologica*, Vol. I, 1956, no. 4)

ÜBER NEMATODENSUKZESSIONEN BEI ZUCKER- UND FUTTERRÜBEN [1])

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Für die Untersuchung von Nematodengemeinschaften, die je nach dem Zersetzungsgrad pflanzlichen Gewebes eine andere Zusammensetzung haben, eignen sich sehr gut knollenartige Gewächse. Sie zeigen die einzelnen Phasen der Umsetzung in deutlich gegeneinander abgegrenzter Form und bieten zugleich eine sich über mehrere Wochen oder gar Monate erstreckende Beobachtungsmöglichkeit.

Für unsere Untersuchungen, die wir über zwei Jahre durchführten, benutzten wir Zucker- und Futterrüben mit einem starken Befall von Stengelälchen (*Ditylenchus dipsaci* (Kühn) Filipjev). Von mehreren Rüben wurden Scheiben geschnitten, diese in grossen Doppelschalen im Labor aufgehoben und von Zeit zu Zeit aus den gesunden und den in Zerfall befindlichen Teilen Proben entnommen. Die Isolierung der Nematoden erfolgte nach dem Baermann-Verfahren. Die so gewonnenen Älchen wurden dann bestimmt.

Bei den für die Untersuchung vorbereiteten Proben unterscheiden wir ausser gesundem Gewebe (Stadium 1) drei Stadien des Gewebezerfalls, nämlich braunes lamellenartiges, trockenfaules Gewebe (Stadium 2), fortgeschrittene Fäule, Lamellenschicht aber noch erkennbar, jedoch noch keine Nassfäule (Stadium 3) und Nassfäule mit wenig geschichtetem Gewebe (Stadium 4).

Die Aufführung aller gefundenen Nematodenarten unter Angabe ihres prozentualen Anteils erübrigt sich, da sich in jedem Falle kleinere Unterschiede zeigten; auch ist das kurzfristige Auftreten bestimmter Nematodenarten nicht ungewöhnlich. Jedoch seien die Leitformen der einzelnen Befallsstufen genannt, die mengenmässig jeweils den grössten Teil der Individuen stellten.

An der Aussenschicht der Rüben finden sich freilebende Nematoden der Gattungen *Eucephalobus* (*E. elongatus* (de Man) Thorne,

1) Eingegangen: 28 April 1956.

E. striatus (Bastian) Thorne, *Cephalobus persegnis* Bastian), *Rhabditis* (*Rh. brevispina* (Claus) Bütschli, *Rh. terricola* Dujardin), *Panagrolaimus* (*P. rigidus* (Schn.) Thorne) und *Dorylaimus* (*D. obtusicaudatus* Bastian), u.a. In dem einwandfrei gesunden Gewebe wurden keine Nematoden ermittelt. Das lamellenartige Stadium 2 war artenarm und enthielt fast ausschliesslich *Ditylenchus dipsaci*, der in einzelnen Kolonien bald stärker, bald weniger stark anzutreffen war. Die Mehrzahl der Tiere fand sich an der Grenzschicht zum gesunden Gewebe. Vorherrschend waren in allen Fällen die Larven. Die Weibchen dringen zuerst in die mittleren Teile der Rübe vor, während die Männchen hier offenbar fehlen. Unter bestimmten Verhältnissen kommt es zu einer Massenvermehrung. Es scheinen sich regelrechte Älchenknäuel zu bilden, wie sie u.a. Hodson (1946) bei lagernden Narzissen beobachtet hat (Abb. 1). Die Tiere sind derart miteinander verschlungen, dass sie sich in Wasser nicht leicht voneinander lösen. Bringt man sie indes in Methylzellulose (1,5 % u. stärker) oder auf ein Filtrierpapier, lösen sich die Knäuel auf. In einem Fall hatten sich in einer Doppelschale, die einige Rübenstückchen in Wasser enthielt, am Deckel über Nacht Flocken von Älchen angesetzt. Die Tiere bewegten sich im Kreise. Nach einer gewissen Zeit lösten sich einzelne Älchen aus dem Kreis. Ihnen folgten weitere, sodass schliesslich „Strassen" sich vorwärts bewegender Älchen entstanden (Abb. 2). Diese „Strassen" verzweigten sich gelegentlich und einzelne Älchengruppen bildeten dann eine neue „Strasse" (Abb. 3). Auch bei Tageslicht hörte das Wandern der Älchen nicht auf. Der erste Infektionsherd dehnte sich bei Zuckerrüben im Labor und in einem kühlen Aufbewahrungsraum nur sehr langsam aus. Überhaupt erfolgte die Zersetzung des pflanzlichen Gewebes bei den Zuckerrüben wesentlich langsamer als bei den Futterrüben, was anscheinend auf die unterschiedlichen osmotischen Verhältnisse in beiden Substraten zurückzuführen ist.

Das Stadium 3 umfasste eine Gemeinschaft mehrerer Nematodenarten, von denen *Panagrolaimus rigidus*, *Aphelenchoides parietinus* (Bastian) Steiner und *Aphelenchus avenae* Bastian den Hauptanteil stellten. Daneben kamen in der Übergangszone auch noch Altlarven von *Ditylenchus dipsaci* vor. So fanden sich in einer Probe noch 45% *Ditylenchus dipsaci* und 53% *Panagrolaimus rigidus*. *P. rigidus* tritt zwar an gesunden Pflanzen und an keimenden Samen auf, findet sich aber auch als Bewohner faulender pflanzlicher Substanzen; anscheinend werden jedoch Stoffe gemieden, die sich bereits weitgehend

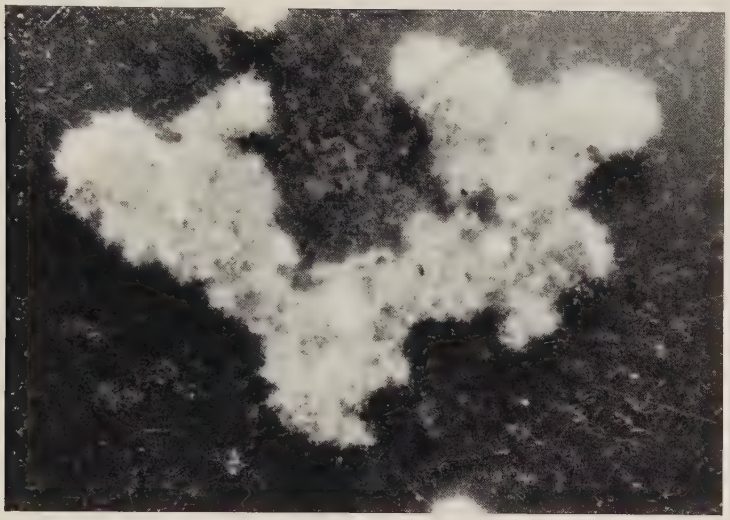

Abb. 1. Massenansammlung von *Ditylenchus dipsaci* („Älchenwolle").

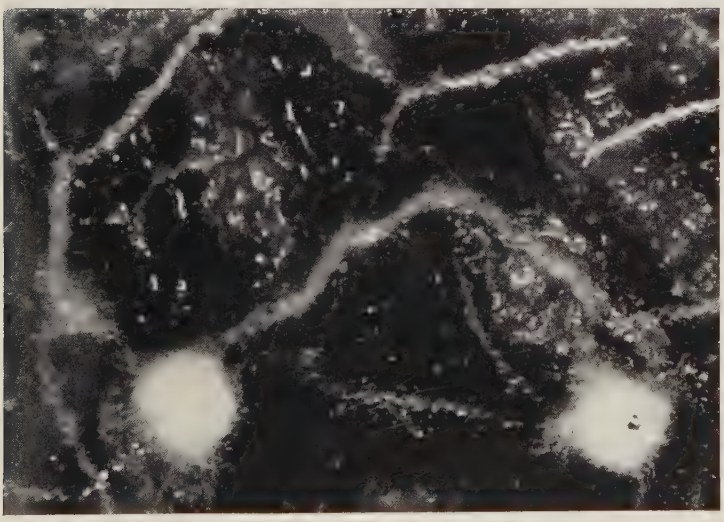

Abb. 2. Flocken von *Ditylenchus dipsaci* unter dem Deckel einer Glasschale mit „Strassen".

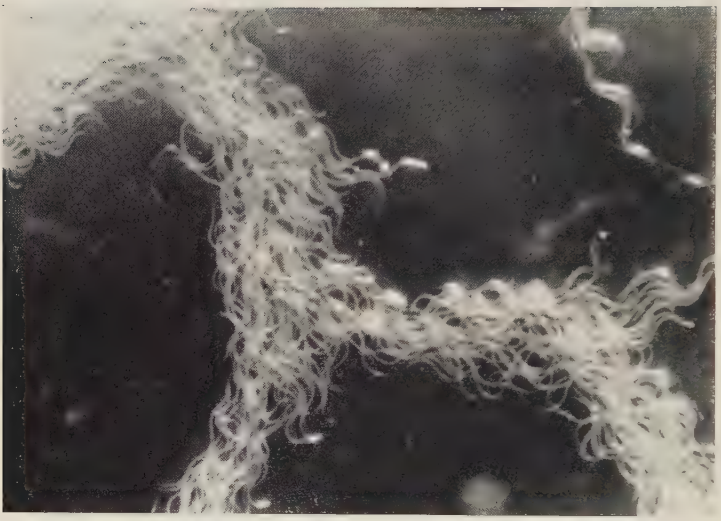

Abb. 3. Vergrösserte Teilansicht einer „Strasse" mit Abzweigung.

zersetzt haben. Es wird angenommen, dass die Art sich weitgehend von Mikroorganismen ernährt, die keine Nassfäule hervorrufen.

Das ubiquitäre Vorkommen und die Ernährungsbreite von *Aphelenchoides parietinus* sind bekannt. Der Nematode parasitiert nicht nur an gesundem Gewebe, sondern ernährt sich auch von sich zersetzenden organischen Stoffen, d.h. wohl hauptsächlich von den hier auftretenden Bakterien und Pilzen, jedoch meidet er ebenfalls nassfaules Gewebe. In Verbindung mit *Aphelenchoides parietinus* tritt häufig *Aphelenchus avenae* auf, dessen nekrobiotische Lebensweise zwar bekannt ist, aber das Gewebe darf einen bestimmten Grad der Zersetzung noch nicht überschritten haben.

Es ist interessant zu beobachten, dass im weiteren Verlauf der Zersetzung nicht nur *Ditylenchus dipsaci* vollkommen verschwindet, sondern dass auch *Aphelenchoides parietinus* und *Aphelenchus avenae* zahlenmässig bedeutend abnehmen, während sich *Panagrolaimus rigidus* noch längere Zeit hält. Wir verzeichnen also mit Zunahme der Zersetzung einen ständigen Rückgang und schliesslich ein völliges Verschwinden der stacheltragenden Arten, wobei bemerkt werden muss, dass Nematoden mit kräftigem Mundstachel vor solchen mit schwach geknöpftem Mundstachel verschwinden. Wenn das Gewebe noch weiter zerfällt, und die ersten Erreger der Nassfäule auftreten (Stadium 4), nimmt auch *P. rigidus* zahlenmässig ab. Statt seiner kommt *Rhabditis brevispina* auf, die bei der starken Vermehrung oft 90% aller gefundenen Nematoden ausmacht. Im Gegensatz zu *Aphelenchoides parietinsus* und *Aphelenchus avenae* ist die Art ein ausgesprochener Bewohner vollfauler Substanzen, aber sie stellt offenbar noch nicht das Endglied dar. Dieses bildet *Diplogaster lheritieri* Maupas, der in allen Entwicklungsstadien das Substrat bevölkert und über ein sehr hohes Vermehrungspotential verfügt.

Die hier aufgeführte Nematodensukzession konnte auch in mehreren anderen Fällen, u.a. auch bei faulenden Kartoffeln beobachtet werden. Man kann damit aus dem Auftreten bestimmter Nematodenarten auf den Zersetzungsgrad des pflanzlichen Gewebes schliessen. Sachs (1950) hat in sich zersetzendem Mist eine ähnliche stets wiederkehrende Reihenfolge in der Besiedlung des Substrats durch bestimmte Nematodenarten festgestellt und glaubt, die Ursache in der Änderung der Bakterien- und Pilzflora des Mistes suchen zu müssen. Osche (1952) konnte experimentell zeigen, dass die grössere oder geringere Fähigkeit zur aktiven Osmoregulation eine weitere Ursache für das Auftreten der einzelnen Nematodenarten darstellt, denn der osmotische

T. Goodey, J. B. Goodey & Mary T. Franklin. *The nematode parasites of plants catalogued under their hosts,* 140 pp. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks., 1956. Price 27 s. 6 d.

"The nematode parasites of plants catalogued under their hosts" by T. Goodey was published in 1940 and has since been an invaluable reference work for host plant studies in this field. The revision has about doubled its size and it now comprises some 4730 records up to end of 1954 extracted from 785 publications.

The body of the work consists of an alphabetical catalogue of the scientific names of plants which have been reported as hosts of nematodes. All the records, the common names and the plant synonyms are now put into a single list. The eelworm names are brought up to date. The general considerations in the Introduction about systematic questions are well worth reading. Where some doubt was felt as to the present identity of an old record, this has been noted with an interrogation mark (about 450 times) or followed by the indication "sic" (about 30 times). This indicates something of the validity of the record, but on the other hand introduces the authors' opinion, which may have its pros and cons.

Some 48 new host plant records of the authors and 76 "in litt." records were inserted in the catalogue. It is the reviewer's opinion that these would have been better omitted in a compilation work, as they may interfere with the proper publication of the records. This is especially the case with polyphagous species of nematodes. It is likely that one has to list nearly all plant species as hosts of certain *Pratylenchus* and other root-attacking species of eelworms, when the presence of reproducing eelworms is considered as evidence.

For such species records are of no value until further data on damage and population increase are published. Indiscriminate recording of host plants of polyphagous nematodes could easily reduce the value of future editions of the book, as is already the case with host-plant lists on insects and moulds.

The publication is very accurate. Only a few records have been overlooked and the number of mistakes, including typographical errors, is small. An exception must perhaps be made for literature in foreign languages, where misprints are not scarce (e.g. about 25 in 50 Dutch references).

However, this does not reduce the value of the book for nematologists and phytopathologists, who will be greatly indebted to the authors if the files are kept up to date for further revisions or additions. M. Oostenbrink

NEMATOLOGICAL NEWS

The author of Nematological News again requests all colleagues to inform him about recent publications on nematology and to send reprints if possible. Although papers eventually appear in The Zoological Record and other abstracting journals, a period of one or two years usually elapses between the publication of the original paper and the appearance of its title or abstract. Therefore we believe that a valuable service is rendered to all nematologists by nothing the titles of papers as soon as possible. We offer our thanks to all those who have co-operated in the first year.

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(*M. javanica* produces rather small galls on squash roots, the diameter of which is two to three times the normal diameter of the roots).

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A. H. Meyl

SOCIETY OF EUROPEAN NEMATOLOGISTS

At the Zoological Congress in 1953 in Copenhagen, the founding of a Society of European Nematologists was suggested. At the third Symposium in Wageningen in 1955, this suggestion was adopted and an executive committee was chosen consisting of the following:—

Ahlberg (Sweden)	Jones (Great Britain)
Bovien (Denmark)	Ritter (France)
Van den Brande (Belgium)	Savary (Switzerland)
Gállego (Spain)	Seinhorst (Netherlands)
Goffart (Germany)	

Secretary: Lindhardt (Denmark)

As it was obvious that a committee of 10 members from 9 different countries would be unpractical and would involve excessive correspondence, a governing board was set up consisting of four officers:

Bovien (Denmark)	Jones (Great Britain)
Goffart (Germany)	Seinhorst (Netherlands)

with Lindhardt (Denmark) as secretary.

The purpose of the Society is to foster collaboration between nematologists, and to call together a symposium every other year. The management of the symposium shall be the duty of the host country.

Membership is open to any person working on plant-parasitic or free-living nematodes whether resident in Europe or elsewhere.

Annual subscriptions will be necessary to defray expenses connected with correspondence. For the present they will be 5 shillings annually. Cheques should be made payable to the Society for European Nematologists, account No. 35668, Lyngby Bank, Lyngby, Denmark.

The Society will publish an annual list of members with their addresses and the subjects on which they at present are working.

A Symposium is being organised by Dr H. Goffart and will take place in Hamburg from September 5-7, 1957. Formal invitations will be sent out by Dr. Goffart early in the New Year and will be limited to those who signify their intention of becoming members of the Society.

K. Lindhardt, Secretary
State Plant Pathology Institute
Lyngby - Denmark

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